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50th THE INTERNATIONAL LIVER CONGRESS™ 2015

Abstract Book

50th Annual Meeting of the European Association for the Study of the Liver
Vienna, Austria - April 22-26, 2015

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50th Annual Meeting of the European Association for the Study of the Liver

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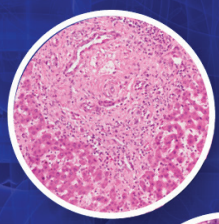

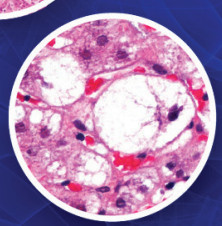

Agenda

PBC and NASH: Serious Liver Diseases with Unmet Needs — *Michael Trauner, MD*

PBC Challenges: What is Treatment Success and What Will Emerging Therapies Offer? — *David E.J. Jones, MD, PhD*

NASH: Diagnostic Challenges, Therapeutic Targets, and New Paths to Treatment Success — *Vlad Ratziu, MD*

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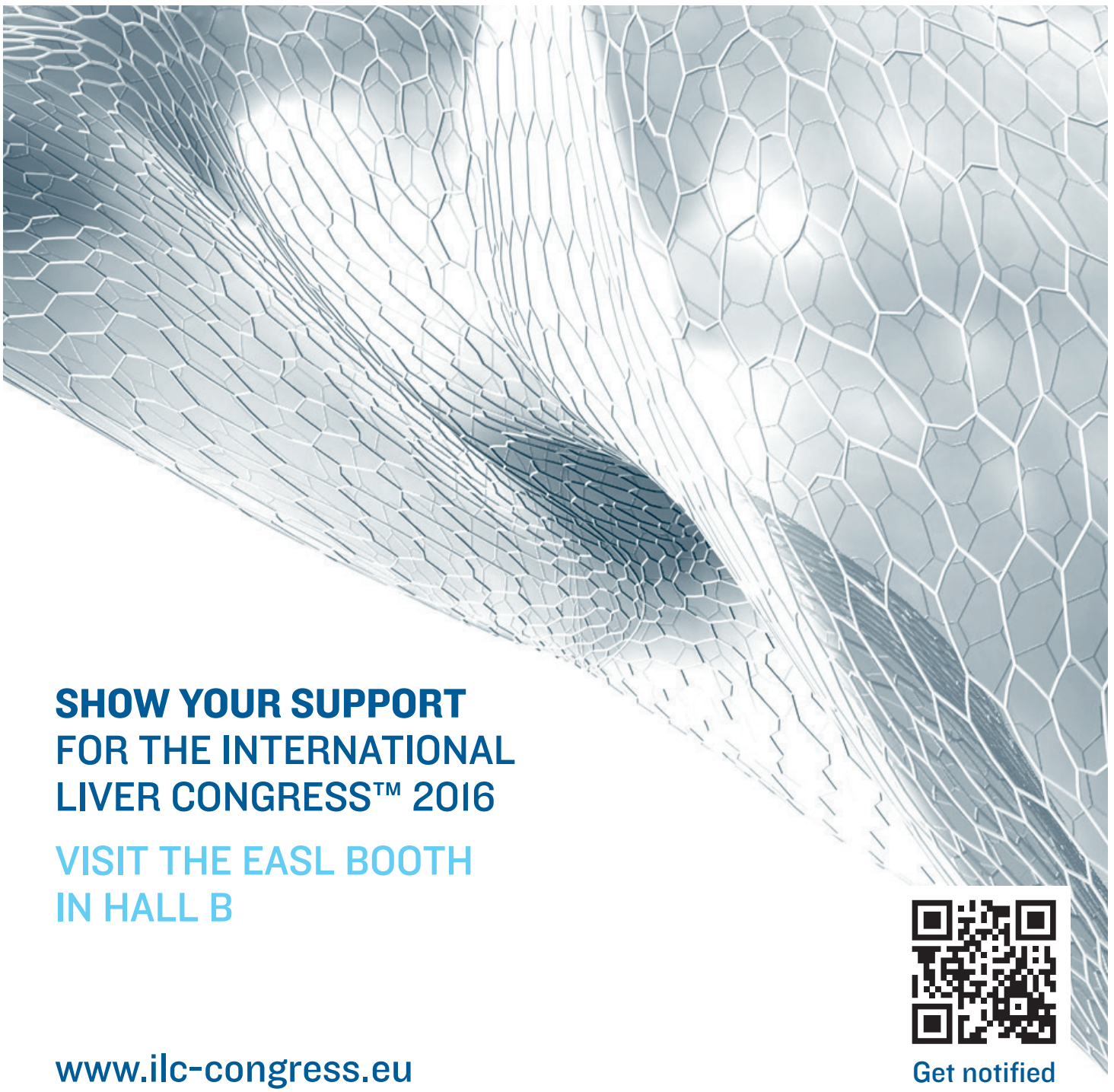

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More detailed information regarding clinical trials and registration can be found in New Engl J Med 2004; 351:1250–1251 and New Engl J Med 2005; 352:2437–2438.

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Thursday, 23 April 2015

General Session 1 & Opening Ceremony

G01

LIRAGLUTIDE IS EFFECTIVE IN THE HISTOLOGICAL CLEARANCE OF NON-ALCOHOLIC STEATOHEPATITIS IN A MULTICENTRE, DOUBLE-BLINDED, RANDOMISED, PLACEBO-CONTROLLED PHASE II TRIAL

M.J. Armstrong[1,2], P. Gaunt[3], G.P. Aithal[4], R. Parker[5], D. Barton[6], D. Hull[1], K. Guo[1], G. Abouda[7], M. Aldersley[8], S.C. Gough[9], J.W. Tomlinson[9], R.M. Brown[10], S.G. Hübscher[10,11], P.N. Newsome[1,2]. *[1]NIHR Liver BRU and Centre for Liver Research, University of Birmingham, [2]Liver Transplant Unit, Queen Elizabeth University Hospital Birmingham, [3]NIHR Liver BRU Clinical trials group (EDD), CRUK clinical trials unit, University of Birmingham, Birmingham, [4]NIHR Nottingham Digestive Diseases Biomedical Research Unit, Nottingham University Hospitals NHS Trust and University of Nottingham, Nottingham, [5]University of Birmingham, Birmingham, United Kingdom; [6]NIHR Liver BRU Clinical trials group (EDD), CRUK clinical trials unit, University of Birmingham, Birmingham, [7]Gastroenterology and Liver Unit, Hull Royal Infirmary, Hull, [8]Liver Transplant Unit, St James Hospital, Leeds, [9]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, [10]Department of Cellular Pathology, Queen Elizabeth University Hospital Birmingham, [11]School of Cancer Sciences, University of Birmingham, Birmingham, United Kingdom*
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Background and Aims: Despite non-alcoholic steatohepatitis (NASH) being the commonest cause of chronic liver disease there are still no licensed therapies. Glucagon-like peptide-1 (GLP-1) analogues are licensed in type 2 diabetes, and are promising therapeutic candidates for NASH, yet no trial data exists on their efficacy in this setting. The histological efficacy and safety of the long-acting GLP-1 analogue, liraglutide, was assessed in patients with NASH.
Methods: In the Liraglutide Efficacy and Action in NASH (LEAN) trial (ClinicalTrials.gov NCT01237119) overweight patients with biopsy-confirmed NASH were randomised (1:1) to receive 48-weeks treatment with once-daily, subcutaneous injections of either 1.8 mg liraglutide or liraglutide-placebo (control). Randomisation was stratified by trial centre (n = 4) and diabetes status. The primary outcome measure as assessed centrally by two independent histopathologists (RMB, SGH) was improvement in liver histology, defined as 'resolution of definite NASH' and no worsening in fibrosis from baseline to end-of-treatment (EOT). Preliminary analyses were performed by intention-to-treat.
Results: Of the 52 patients randomised (mean age 51 years, 60% male, 33% type 2 diabetes, 52% F3/F4 Kleiner fibrosis stage), 45 patients underwent EOT liver biopsies. The primary end-point was met, and 9 (39%) of 23 patients on liraglutide had resolution of definite NASH compared to 2 (9%) of 22 patients on placebo (p = 0.019). Only 2 (9%) patients on liraglutide had worsening of fibrosis compared to 8 (36%) on placebo (p = 0.026). Moreover, liraglutide reduced weight (−5.3 vs. −0.6 kg, p = 0.001), BMI (−1.8 vs. −0.3 kg/m^2, p = 0.003), and fasting glucose (−1.0 vs −0.7 mmol, p = 0.005) compared to placebo. Reductions in ALT (−27 vs. −10, p = 0.126) and HbA1c (−0.5% vs. −0.03, p = 0.07) were also seen with liraglutide, albeit not significant versus placebo. There were no treatment differences in lipid profile or systolic BP. There were no serious adverse events in patients on liraglutide, which was well-tolerated with only 2 (8%) of 26 patients withdrawing from treatment due to drug-related gastro-intestinal (nausea, diarrhoea) side effects.
Conclusions: In a randomised controlled trial liraglutide met the primary end-point of histological clearance of NASH, and a reduction in progression of fibrosis. It was also safe and well-tolerated. Phase III registration trials are now warranted for GLP-1 therapy in patients with NASH.

G02

LEDIPASVIR/SOFOSBUVIR WITH RIBAVIRIN IS SAFE AND EFFICACIOUS IN DECOMPENSATED AND POST LIVER TRANSPLANTATION PATIENTS WITH HCV INFECTION: PRELIMINARY RESULTS OF THE PROSPECTIVE SOLAR 2 TRIAL

M. Manns[1], X. Forns[2], D. Samuel[3], J. Denning[4], S. Arterburn[4], T. Brandt-Sarif[4], H. Dvory-Sobol[4], P.S. Pang[4], J.G. McHutchison[4], E. Gane[5], D. Mutimer[6]. *[1]Hannover Medical School, Hannover, Germany; [2]Liver Unit, IDIBAPS, CIBEREHD Hospital Clinic, Barcelona, Spain; [3]Université Paris-Sud, Villejuif, France; [4]Gilead Sciences, Inc., Foster City, United States; [5]University of Auckland, Auckland, New Zealand; [6]QE Hospital and University of Birmingham, Birmingham, United Kingdom*
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Background and Aims: Treatment options for patients with chronic hepatitis C (HCV) who have decompensated liver disease or who have undergone liver transplantation are limited. We evaluated the safety and efficacy of ledipasvir/sofosbuvir (LDV/SOF) fixed dose combination with ribavirin (RBV) in such patients in Europe, Canada, Australia and New Zealand.
Methods: We enrolled HCV genotype 1 or 4 treatment-naïve or treatment-experienced patients with decompensated liver disease or post-liver transplantation with recurrent HCV. Patients were randomized to receive 12 or 24 weeks of treatment stratified into 6 groups: patients without transplant and either (1) Child–Pugh–Turcotte (CPT) B cirrhosis, or (2) CPT C cirrhosis; or patients with recurrent HCV after liver transplantation and were either (3) without cirrhosis (F0 to F3), (4) CPT A cirrhosis, (5) CPT B cirrhosis, or (6) CPT C cirrhosis.
Results: 327 patients were randomized. Most were male (76%), white (94%), and IL28B non-CC (80%). 159 (49%) had HCV genotype (GT) 1a, 131 (40%) GT 1b, and 37 (11%) GT 4. Mean baseline HCV RNA was 6.2 $\log_{10}$ IU/mL. 41 of 227 cirrhotic patients (18%) had a MELD score >15. 222 (68%) patients have completed study treatment and 171 (52%) have reached post treatment week 4, so far. Nine patients in the advanced liver disease group and 3 patients in the post-transplantation group have discontinued study treatment. 62 patients (19%) experienced serious adverse events (SAEs). Eight SAEs in 8 patients were considered related to study treatment; anemia (4), fall, diarrhea, malaise, and hyperbilirubemia. The

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Table (abstract G02).

	Advanced liver disease pre-transplantation				Recurrent HCV post liver transplantation							
	CPT B		CPT C		F0–F3		CPT A		CPT B		CPT C	
	12 wks Rx (N = 29)	24 wks Rx (N = 29)	12 wks Rx (N = 24)	24 wks Rx (N = 25)	12 wks Rx (N = 51)	24 wks Rx (N = 49)	12 wks Rx (N = 34)	24 wks Rx (N = 33)	12 wks Rx (N = 22)	24 wks Rx (N = 22)	12 wks Rx (N = 3)	24 wks Rx (N = 6)
SVR4	24/28 (86)	11/11 (100)	14/16 (88)	3/6 (50)	29/31 (94)	8/8 (100)	31/32 (97)	11/12 (92)	17/17 (100)	6/6 (100)	2/2 (100)	1/2 (50)

most common adverse events were fatigue, anemia, nausea and headache. SVR4 by patient population are presented in the table.
Conclusions: Administration of LDV/SOF+RBV in patients with decompensated cirrhosis and recurrent HCV post transplantation has been well tolerated and resulted in high SVR4 rates in these very difficult to treat populations treated with either 12 or 24 weeks of this regimen. SVR 12 results will be presented.

G03
RIFAXIMIN AND PROPRANOLOL COMBINATION THERAPY IS MORE EFFECTIVE THAN PROPRANOLOL MONOTHERAPY IN THE HEPATIC VENOUS PRESSURE GRADIENT RESPONSE AND PROPRANOLOL DOSE REDUCTION – A PILOT STUDY

S.K. Baik[1], Y.L. Lim[1], Y.Z. Cho[1], M.Y. Kim[1], Y.O. Jang[2], K.T. Suk[3], G.J. Cheon[4], Y.D. Kim[4], D.H. Choi[5]. *[1]Internal Medicine, [2]Cell Therapy and Tissue Engineering, Wonju Christian Hospital, Wonju, [3]Internal Medicine, Chuncheon Sacred Heart Hospital, Chuncheon, [4]Internal Medicine, Kangneung Asan Hospital, Kangneung, [5]Internal Medicine, Kangwon University School of Medicine, Chuncheon, Korea, South*
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Background and Aims: Nonselective beta blocker is the only regimen accepted to reduce the portal pressure. However its anti-portal pressure effect is insufficient in many cases and the side effects have made a limitation in clinical use. Gut bacterial translocation and production of endotoxin is known to increase portal pressure in cirrhosis. So, this study investigated the effect of addition of gut decontamination therapy using non-absorbable antibiotics, rifaximin to nonselective beta blocker, propranolol.
Methods: We included 65 patients of advanced cirrhosis from January 2011 to July 2013 and randomly assigned propranolol monotherapy (n = 48) and propranolol and rifaximin combination therapy (n = 17). For monotherapy group, propranolol dose was titrated to maximum 320 mg/day with a target of 25% heart rate (HR) reduction. In combination group, propranolol dose was also titrated according to the HR however, the maximum dose was limited to 160 mg/day and rifaximin 1200 mg/day was administered. Both baseline and treatment 3months later hepatic venous pressure gradient (HVPG) were measured in all patients. Patients with HVPG reduction by ≥20% or to less than 12 mmHg were defined as responders. Mean blood pressure (MBP) and HR, side effects and other serologic data were also collected.
Results: Portal pressure declined significantly in both groups (propranolol monotherapy group 17.0±3.9 mmHg to 13.5±4.1 mmHg, combination group 16.7±3.6 mmHg to 10.9±4.7 mmHg, each $P < 0.001$). Combination group showed better HVPG response rate than monotherapy group (82.4% vs. 50.0%, $P = 0.018$) and the mean reduction of HVPG was also larger in combination group (5.8±3.8 mmHg vs. 3.5±3.9 mmHg, $P = 0.038$). Especially, the mean dose of propranolol (152±59.3 mg vs. 127.0±32.4, $P = 0.033$) and the reduction of HR (20.5±13.0% mg vs. 7.4±15.5%, $P = 0.001$) were smaller in combination group. The reduction of MBP did not show difference between two groups (4.4±12.3 mmHg vs. 3.1±11.7 mmHg, $P = 0.695$). Dizziness related with orthostatic hypotension was observed in 8 (mono) and 2 (combination) cases of each group.
Conclusions: Propranolol and rifaximin combination therapy showed additive effect in the reduction of HVPG with smaller dose of propranolol and side effects and it suggests that addition of rifaximin can be a good solution to break the limitations of non-selective beta blocker.

G04
ENGINEERED HBV-SPECIFIC T CELLS: DISENTANGLING ANTIVIRAL FROM KILLING CAPACITY

S. Koh[1], C.Y.L. Tham[2], A.T. Tanoto[2], A. Pavesi[3], R.D. Kamm[4], A. Bertoletti[2]. *[1]Singapore Institute for Clinical Sciences A*STAR, [2]Emerging Infectious Diseases Program, Duke-NUS Graduate Medical School, [3]Singapore MIT Alliance – SMART, Singapore, Singapore; [4]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, United States*
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Background and Aims: We have recently demonstrated that adoptive transfer of engineered HBV-specific T cells in a patient with HBsAg-productive HCC cause a profound inhbition of HBsAg production (J Hepatology 2014. doi: 10.1016/j.jhep.2014.10.001.). The difficulty to disentangle the antiviral effect of HBV T cells from their killing ability constitutes a barrier to the application of this immunotherapeutic approach in chronic HBV patients. Therefore, our aim was to produce HBV-specific T cells that can inhibit virus replication without hepatotoxicity and analyzed the antiviral mechanism mediated by these engineered T cells.
Methods: Using messenger RNA electroporation of HBV-T cell receptor in human lymphocytes, we produced HBV-specific T cells characterized by different activation and maturation stages. These distinct HBV-specific T cell populations were tested in 2 dimensional (2D) and 3D in vitro assays for their ability to inhibit HBV replication and/or lyse target cells.
Results: Among different engineered HBV-specific T cell populations, we selected a perforin/granzymelow HBV-specific T cells able to induce 50% drop in HBV viral load in HepG2.2.15 without causing detectable hepatotoxicity at an effector to target ratio of 1:3 (both in 2D and 3D models). After HBV-specific recognition, these resting naïve (CD28+CD27+) T cells produced a large range of cytokines (IFN-gamma, TNF-alpha, GM-CSF and lymphotoxin-alpha and -beta). Analysis of the specific antiviral mechanisms indicated that a specific synergy between classical antiviral cytokines (IFN-gamma, TNF-alpha, GM-CSF) and activator of lymphotoxin-β receptor (LTβR) pathway mediate the antiviral but non-cythopathic effect.
Conclusions: It is possible to produce HBV-specific T cells able to efficiently inhibit HBV replication without causing direct hepatocytes killing. This represents an attractive cell population for adoptive T cell therapy of chronic hepatitis B. The relative contribution of antiviral cytokines and/or LTβR activation to antiviral activity can lead to the development of new targeted combinatorial therapy in HBV infection.

G05

EXOME SEQUENCING OF 243 LIVER TUMORS IDENTIFIES NEW MUTATIONAL SIGNATURES AND POTENTIAL THERAPEUTIC TARGETS

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Background and Aims: Hepatocellular carcinoma (HCC) is a heterogeneous disease mainly developing in chronic liver disease and caused be a variety of etiologies. Hepatocarcinogenesis is a multi-step process in which pre-cancerous lesions can ultimately transform into liver cancer. Genomic analyses promise to unravel the mutagenic processes in order to identify molecular profiles, linked to aggressiveness of the tumor, and to optimize personalized patient care.

Methods: We performed exome sequences of 243 liver tumors from European patients (France, Italy and Spain), associated with cirrhosis (F4, n = 118), fibrosis (F2+F3, n = 46), or non-fibrotic livers (F0+F1, n = 79). Cirrhosis-associated tumors presented different stages along HCC progression: 7 dysplastic macronodules, 7 early, 17 small and progressed, 58 classic, and 29 poor-prognosis HCC. Tumors were categorized by varying risk factors: Significant alcohol intake (41%), HCV (26%), non-alcoholic steatohepatitis (18%), HBV (14%), hemochromatosis (7%), no known etiology (11%).

Results: We identified 8 mutational signatures of which 6 (Signatures 1A, 1B, 4, 5, 6, and 16) were previously validated in a pan-cancer analysis, while 2 (Signatures 23 and 24) are novel. Hierarchical clustering, based on mutational signatures, revealed 6 groups (MSig1 to 6) and 4 singletons which were significantly associated with specific risk factors, mainly combined alcohol/tobacco consumption, and aflatoxin B1. Next, we identified 161 putative driver genes associated with 11 recurrent pathways. Associations of mutations defined 3 groups of genes centered on *CTNNB1* (alcohol), *TP53* (HBV), and *AXIN1*. Analyses according to tumor stage progression revealed *TERT* promoter mutation as an early event whereas *FGF/CCND1* amplification, *TP53* and *CDKN2A* alterations, appeared at more advanced stages in aggressive tumors. Altogether, 28% of patients harbored at least one damaging alteration potentially targetable by an FDA-approved drug and 86% by a drug which has been studied in phase I to phase III clinical trials.

Conclusions: In conclusion, our study identified relationships between environmental exposures and mutational patterns in HCC as well as the landscape of driver genes and pathways altered in different clinical stages and etiological backgrounds. For patient care, genomic alterations identified in targetable genes will be useful to determine HCC patients that could potentially benefit from targeted treatment in future clinical trials.

G06

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE III TRIAL OF TSU-68 (ORANTINIB) COMBINED WITH TRANSCATHETER ARTERIAL CHEMOEMBOLIZATION IN PATIENTS WITH UNRESECTABLE HEPATOCELLULAR CARCINOMA

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Background and Aims: Transcatheter arterial chemoembolization (TACE) is considered an effective treatment for unresectable hepatocellular carcinoma (HCC). However, TACE hardly achieves complete tumor necrosis and potentially causes tumor hypoxia inducing angiogenesis. TSU-68 is an oral tyrosine-kinase inhibitor of vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptors. In a phase II study, TSU-68 treatment combined with a single TACE session tended to prolong progression-free survival compared to TACE alone (Inaba et al. 2013). Herein, we present our findings from a randomized, double-blind, placebo-controlled phase III study (ORIENTAL) to evaluate the effect of concurrent TSU-68 combined with conventional TACE (cTACE).

Methods: Patients were randomly assigned (1:1) within 28 days from receiving TACE according to their Child–Pugh score, serum alpha-foetoprotein level, and intrahepatic nodule size. TSU-68 or placebo was administered orally at 200 mg, twice per day, and continued until TACE failure or unacceptable toxicities were observed. Two levels of dose modifications (200/0 daily and 200/0 every other day) were permitted. The primary endpoint was overall survival (OS), and secondary endpoints included time to TACE failure (TTTF), safety profile, and biomarker analysis.

Results: A total of 889 patients were enrolled from Japan, Korea, and Taiwan. Patient characteristics were well balanced. The study was terminated because interim analysis showed no OS improvement by TSU-68 compared to placebo (31.1 vs. 32.3 months, p = 0.435), despite a trend towards prolonged median TTTF (23.9 vs. 19.8 months, p = 0.245). In stratified analysis, the median TTTF in the TSU-68 arm was significantly prolonged in patients with low VEGF-C (25.5 vs. 18.4 months, p = 0.0196) and Barcelona Clinic of Liver Cancer (BCLC) stage-B HCC (22.1 vs. 14.9 months, p = 0.054). The main adverse events of TSU-68 were oedema, ascites, and elevation of aspartate and alanine aminotransferases. Most of these did not affect TSU-68 treatment duration, which was 332.0 days. Only one patient died of treatment-related hepatic failure.

Conclusions: TSU-68 combined with repeated cTACE did not improve OS. However, favourable TTTF was observed in patients with low VEGF-C and those with BCLC-B HCC receiving TSU-68. Further study is needed to confirm the potential of VEGF-C as a predictive marker. TSU-68 treatment was tolerable in HCC patients receiving repeated TACE with a high safety profile and long treatment duration of 1 year.

Viral hepatitis C: Therapy

O001

C-SALVAGE: GRAZOPREVIR (GZR; MK-5172), ELBASVIR (EBR; MK-8742) AND RIBAVIRIN (RBV) FOR CHRONIC HCV-GENOTYPE 1 (GT1) INFECTION AFTER FAILURE OF DIRECT-ACTING ANTIVIRAL (DAA) THERAPY

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Background and Aims: Treatment options are needed for patients who do not achieve SVR on regimens containing DAAs. The Phase-2 C-SALVAGE study investigated the safety and efficacy of an interferon-free combination of GZR [NS3/4A protease inhibitor (PI)] and EBR [NS5A inhibitor] with RBV for patients with chronic HCV GT1 infection who had failed licensed DAA-containing therapy.
Methods: C-SALVAGE is an international open-label study of GZR 100 mg QD, EBV 50 mg QD, and weight-based RBV BID for 12 weeks in patients with chronic HCV GT1 infection who had failed ≥4 weeks of peginterferon and RBV combined with boceprevir, telaprevir, simeprevir, or sofosbuvir. Per protocol, ~80% of the enrolled subjects were to have experienced virologic failure. Exclusion criteria included decompensated liver disease, hepatocellular carcinoma, HIV or HBV co-infection, thrombocytopenia $<50\times10^3/\mu L$, or hypoalbuminemia <3.0 g/dL. HCV RNA levels were measured by COBAS TaqMan v2.0 assay. Resistance-associated variants (RAVs) were identified at baseline by population sequencing. The primary efficacy outcome was a HCV RNA level below the limit of quantification (15 IU/mL) 12 weeks after the end of treatment (SVR_{12}).
Results: 79 patients were treated with ≥1 dose of study drug (Table): 33 (42%) were women, 2 (3%) were non-white, 34 (43%) had cirrhosis (including 8 diagnosed by biopsy), 30 (38%) had GT1a infection, and 66 (84%) had a history of virologic failure on a prior DAA-containing regimen [13 (16%) had failed for other reasons]. All had received a PI; none had received sofosbuvir. At entry, 30 (41%) of the 73 patients with available NS3 sequencing data harbored RAVs. 78 (99%) patients completed therapy with 1 early discontinuation due to an AE. At the end of therapy, RNA levels were <15 IU/mL in all 79 (100%) patients. 5 serious AEs (bacterial pharyngitis, laryngeal squamous cell carcinoma, asthma, appendicitis, and urinary tract infection) were reported, all of which were considered unrelated to study drugs.
Conclusions: In the ongoing C-SALVAGE trial, 79 HCV GT1-infected patients who had failed PI-based regimens were treated with GZR/EBR/RBV, including 43% with cirrhosis and 84% with prior virologic failure. HCV RNA levels <15 IU/mL were achieved in all patients at the end of treatment despite a high prevalence of NS3 RAVs at baseline. Study medications were well tolerated in this population. SVR_{12} rates, full safety data, and expanded resistance results will be presented.

Table: Baseline characteristics of 79 patients treated with ≥1 dose of study drug.

Age, yrs	
Mean (Median)	54.4 (55)
Mean BMI, kg/m^2 (SD)	28.0 (4.6)
HCV Genotype, n (%)	
1a	30 (38.0)
1b	49 (62.0)
Fibrosis Stage, n (%)	
F4 (cirrhosis)	34 (43.0)
F3	8 (10.1)
F0–2	37 (46.8)
Screening HCV RNA	
Mean, log_{10}IU/mL (SD)	6.1 (0.5)
DAA Experience, n (%)	
Boceprevir	28 (35.4)
Telaprevir	43 (54.4)
Simeprevir	8 (10.1)
Virologic Failure, n (%)	66 (83.5)
Nonresponse to P-R + DAA	15 (19.0)
Breakthrough on P-R + DAA	9 (11.4)
Breakthrough on P-R tail after DAA	16 (20.2)
Relapse after P-R + DAA	26 (32.9)

O002

TREATMENT OF DECOMPENSATED HCV CIRRHOSIS IN PATIENTS WITH DIVERSE GENOTYPES: 12 WEEKS SOFOSBUVIR AND NS5A INHIBITORS WITH/WITHOUT RIBAVIRIN IS EFFECTIVE IN HCV GENOTYPES 1 AND 3

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Introduction: All oral antiviral therapy for patients with HCV induced decompensated cirrhosis is now possible. The optimal regimen is unclear, particularly for Genotype 3, and debate continues on which patients benefit. The NHS England Early Access Program (EAP) provided 12 weeks of therapy with sofosbuvir, with or without ribavirin and an NS5A inhibitor to a cohort of ~500 patients with decompensated cirrhosis. Here we evaluate viral clearance and safety in the initial 465 patients. This is the first analysis of a large cohort of patients with decompensated cirrhosis due to genotype 3 HCV receiving sofosbuvir plus NS5A inhibitors.
Material and Methods: The EAP allowed physicians to treat patients with decompensated cirrhosis with sofosbuvir combined with either daclatasvir or ledipasvir +/− ribavirin (NS5A inhibitors kindly provided prior to license by Gilead and BMS). Choice of therapy was at the clinicians' discretion. Data and samples were collected by HCV Research UK and we present data on those patients due to have reached 4 weeks post therapy by abstract submission; all were enrolled when daclatasvir was freely available.
Results: 17 centres enrolled 465 patients between July and October 2014, 23 were co-infected with HIV. The Table shows the results and indicates response rates of >80% in patients receiving ribavirin in addition to sofosbuvir and an NS5A inhibitor. For Genotype 3 HCV with decompensated cirrhosis both daclatasvir and ledipasvir

were effective with a numerical increase in patients achieving SVR4 with daclatasvir, although this was not statistically significant. 91 patients required reduction in ribavirin dosing.
19 patients (4%) died during the study (11 on therapy), 16 (3.4%) required transplantation and 181 (39%) had an SAE, which was related to therapy in 143 patients (31%). In 19 patients (4%) liver function deteriorated (worsening ascites/encephalopathy/new variceal bleed) but in 215 patients (46%) ascites/encephalopathy improved.
Conclusions: In decompensated cirrhosis 12 weeks therapy with sofosbuvir plus an NS5A inhibitor is effective with most patients achieving SVR4. Patients infected with Genotype 1 HCV respond well with >80% achieving SVR4, response rates were slightly reduced in patients with G3. In this fragile population with end stage liver disease deaths and progression of liver disease were not uncommon but nearly half the patients had improvements in liver function.

Table: SVR4 rates in patients with decompensated cirrhosis receiving 12 weeks therapy with different treatment regimes

	SOF/LDV	SOF/LDV/Riba	SOF/DCV	SOF/DCV/Riba
Overall	21/28 (75%)	212/251 (84%)	12/15 (80%)	142/171 (83%)
Genotype 1	17/21 (81%)	144/163 (88%)	4/5 (80%)	40/45 (89%)
Genotype 3	4/7 (57%)	44/61 (72%)	5/7 (71%)	92/113 (81%)
Other genotypes	0	24/27 (89%)	3/3 (100%)	10/13 (77%)

Numbers are SVR4/total available for analysis (%).

O003
CAN HEPATITIS C TREATMENT BE SAFELY DELAYED? EVIDENCE FROM THE VETERANS ADMINISTRATION HEALTHCARE SYSTEM

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Background and Aims: The cost of new HCV treatments leads payers and insurance providers to question if delaying treatment for low risk patients can be accomplished without adversely impacting the patient. Retrospective patient data from the Veterans Administration [VA] were used to estimate the impact on patient risk of treatment initiation before and after the patient's FIB4 levels became elevated.
Methods: VA HCV patients with one or more reported FIB-4 values were selected. Primary outcome measures were time to death and time to the first occurrence of a composite of liver-related clinical events. The impact of time to treatment initiation and time to three different definitions of an elevated FIB4 level were estimated using a time-dependent Cox proportional hazards models.
Results: 187,860 patients met study requirements. Initiating treatment before FIB4 >1.00 reduced morbidity by 41% and death by 36%. Initiating treatment after FIB4 >1.00 remained effective but diminished in the morbidity risk reduction achieved [30%]. This is not the case if treatment initiation is delayed until after FIB4 >3.25. The risk reductions associated with treatment initiation before FIB4 >3.25 were 34% for the composite event and 45% for death but if initiated after FIB4 >3.25 were only 11% and 25%, respectively. These detrimental effects of delaying treatment until FIB4 >3.25 were due to a reduction in the likelihood that treated patients would achieve viral load suppression and a reduced impact of viral load suppression on morbidity.
Conclusions: Delaying treatment until after a patient's FIB4 level exceeds 3.25 has a clear detrimental effect on treatment effectiveness

O004
ON-TREATMENT VIROLOGIC RESPONSE AND TOLERABILITY OF SIMEPREVIR, DACLATASVIR AND RIBAVIRIN IN PATIENTS WITH RECURRENT HEPATITIS C VIRUS GENOTYPE 1b INFECTION AFTER ORTHOTOPIC LIVER TRANSPLANTATION (OLT): INTERIM DATA FROM THE PHASE II SATURN STUDY

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Background and Aims: Simeprevir (SMV) and daclatasvir (DCV) are approved for the treatment of hepatitis C virus (HCV) infection in the non-transplant setting. SMV is a NS3/4A protease inhibitor and DCV is a NS5A replication complex inhibitor. The combination of SMV+DCV±ribavirin (RBV) has been previously evaluated in treatment-naïve and prior null responder patients (pts; LEAGUE-1). SATURN is an ongoing open-label Phase II study, investigating SMV+DCV+RBV in pts with recurrent HCV genotype 1b infection after orthotopic liver transplantation (OLT). Virologic response and tolerability results are presented from a pre-planned interim analysis.
Methods: Post-OLT treatment-naïve or -experienced (prior relapser, partial or null responder to peginterferon±RBV) adults on stable immunosuppressive therapy following OLT were included. Part 1 (P1) included pts with METAVIR score F1–F2 on cyclosporine (CsA) or tacrolimus (TAC). Part 2 (P2) included F1–F4 pts on TAC. Pts received SMV (150 mg once daily [QD]), DCV (60 mg QD) and RBV (1–1.2 g/day) for 24 weeks. At the time of analysis, all P1 pts had reached end of treatment (EOT) and in P2, Week 4 of treatment (or early discontinuation). Analyses were based on the intent-to-treat population.
Results: A total of 21 (P1) and 14 (P2) pts were included (P1/P2: female, 33%/43%; median age, 63.0 years [y]/59.5 y; mean time since transplantation, 3.98 y/5.36 y; median baseline HCV RNA 6.9 $\log_{10}$/6.9 $\log_{10}$ IU/mL; METAVIR F3/F4, 0%/57%). P1 CsA pts had ~6-fold higher SMV plasma concentrations leading to SMV dose adjustment and exclusion of CsA pts from P2. On-treatment virologic response is shown in the Table. In P1, 91% of pts had HCV RNA <25 IU/mL undetectable at EOT. In P2, 93% had HCV RNA <25 IU/mL detectable/undetectable at Week 4. The most common adverse events (AEs) were anaemia (P1/P2: 62%[CsA: 70%/TAC: 55%]/21%) and asthenia (P1: 38%). Grade 3/4 AEs were reported in 19% (P1) and 36% (P2) of pts. Serious AEs were reported in 24% and 14% of pts, while 2 P1 and 0 P2 pts discontinued at least one study drug due to an AE. RBV dose reductions occurred in 3 P1 and 2 P2 pts. Grade 3/4 haemoglobin decreases and hyperbilirubinemia were observed in 5% and 33% of P1 and 7% and 36% of P2 pts.

Table (abstract O004).

On-treatment response ITT (n/N, %)	Simeprevir (150 mg) + daclatasvir (60 mg) + ribavirin					
	Week 4 (n = 35)			End of treatment (n = 21)		
	Part 1		Part 2	Part 1		
	Cyclosporine (n = 10)	Tacrolimus (n = 11)	Tacrolimus (n = 14)	Cyclosporine (n = 10)	Tacrolimus (n = 11)*	Total (n = 21)
<25 IU/mL undetectable/detectable	10/10 (100)	10/11 (90.9)	13/14 (92.9)	10/10 (100)	9/11 (81.8)	19/21 (90.5)
<25 IU/mL undetectable**	7/10 (70.0)	8/11 (72.7)	9/14 (64.3)	10/10 (100)	9/11 (81.8)	19/21 (90.5)

ITT, intent-to-treat.
*Includes 1 HCV genotype 1a patient who was enrolled in error and who experienced viral breakthrough.
**In Part 2, this is RVR (rapid virologic response at Week 4 while on treatment).

Conclusions: SMV+DCV+RBV was generally well tolerated and led to a significant on-treatment response in post-OLT pts on immunosuppressive therapy, showing promise as a potential treatment option for the post-OLT pt population.
Study funded by Janssen.

O005
RETREATMENT OF PATIENTS WHO FAILED 8 OR 12 WEEKS OF LEDIPASVIR/SOFOSBUVIR-BASED REGIMENS WITH LEDIPASVIR/SOFOSBUVIR FOR 24 WEEKS

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Background and Aims: Ledipasvir/sofosbuvir (LDV/SOF) fixed-dose combination is the first approved interferon- and ribavirin-free treatment for genotype 1 HCV-infected patients. Of the 1952 patients in LDV/SOF Phase 3 clinical trials, 35 (2%) were virologic failures following 8 or 12 weeks of treatment. This study evaluated the efficacy of LDV/SOF for 24 weeks as retreatment for these patients and other patients that have failed similar regimens in LDV/SOF Phase 2 studies.
Methods: This open-label study enrolled genotype 1 HCV-infected patients who relapsed following 8 or 12 weeks of LDV/SOF±ribavirin (RBV) in Phase 2/3 studies. NS5A and NS5B resistance associated variants were evaluated by deep sequencing. The primary endpoint was SVR12.
Results: 41 patients were enrolled and completed treatment. The mean age was 58 years, 83% male, 24% African-American, 46% had cirrhosis, 93% IL28B CT/TT genotype, 83% GT1a, and mean baseline HCV RNA was 6.2 log10 IU/mL. The overall SVR4 rate was 73% (30/41): 1 breakthrough, 9 relapse, and 1 visit pending. Table 1 presents the SVR4 rates by prior treatment. Headache was the only AE reported in ≥10% of patients. Two patients (5%) had treatment-emergent SAEs. Complete SVR12 rates and viral sequencing results will be presented.

Table 1. SVR4 according to prior treatment

Patients, n/N (%)	LDV/SOF+GS-9669	LDV/SOF±RBV	
	8 weeks (n = 8)	8 weeks (n = 22)	12 weeks (n = 11)
SVR4	7/8 (88%)	18/22 (82%)	5/11 (45%)
95% CI	47–100%	60–95%	17–77%
Breakthrough	0	0	1/11 (9%)
Relapse	1/8 (13%)	4/22 (18%)	4/10 (40%)
Visit pending	0	0	1/11 (9%)

Conclusions: Ledipasvir/sofosbuvir for 24 weeks was well tolerated and demonstrated that successful retreatment is possible in some genotype 1-infected, NS5A-failure patients.

O006
C-SWIFT: GRAZOPREVIR/ELBASVIR + SOFOSBUVIR IN CIRRHOTIC AND NONCIRRHOTIC, TREATMENT-NAIVE PATIENTS WITH HEPATITIS C VIRUS GENOTYPE 1 INFECTION, FOR DURATIONS OF 4, 6 OR 8 WEEKS AND GENOTYPE 3 INFECTION FOR DURATIONS OF 8 OR 12 WEEKS

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Background and Aims: The safety and efficacy of several oral direct-acting antiviral (DAA) regimens for 8–24 weeks has recently been demonstrated. Shorter durations of therapy using novel combinations of DAAs may offer benefits such as increased compliance, lower cost and broader usage and acceptance by treating providers. The aim of this study was to assess the efficacy and safety of grazoprevir (GZR;NS3/4A protease inhibitor) and elbasvir (EBR; NS5A inhibitor) fixed-dose combination (FDC) + sofosbuvir (SOF; nucleotide NS5B polymerase inhibitor) in treatment-naive patients with and without cirrhosis with hepatitis C virus (HCV) genotype 1 (G1) infection for shortened durations of 4, 6 or 8 weeks and G3 infection for durations of 8 or 12 weeks.
Methods: C-SWIFT enrolled 102 G1 and 41 G3 treatment-naive patients with and without cirrhosis. G1 noncirrhotic (n = 61) patients were randomized to 4 or 6 week durations; G1 cirrhotic (n = 41) patients were randomized to 6 or 8 weeks of therapy. G3 noncirrhotic patients (n = 29) were randomized to 8 or 12 weeks and G3 cirrhotic patients (n = 12) were assigned to 12 weeks of therapy. All patients received the same regimen: GZR/EBR (100 mg/50 mg) FDC + SOF (400 mg). HCV RNA was assessed using COBAS TaqMan v2.0 (LoQ 15 IU/mL). The primary end point is the proportion of patients achieving HCV RNA <15 IU/mL 12 weeks after end of treatment (SVR12).

	G1				G3		
	Noncirrhotic		Cirrhotic		Noncirrhotic		Cirrhotic
Treatment duration	4 weeks	6 weeks	6 weeks	8 weeks	8 weeks	12 weeks	12 weeks
Randomized, N	31	30	20	21	15	14	12
TW4, % (n/m[a])	81% (25/31)	83% (25/30)	90% (18/20)	90% (19/21)	93% (14/15)	86% (12/14)	75% (9/12)
SVR2, % (n/m[a])	100% (31/31)	100% (30/30)	100% (20/20)	100% (19/19)	100% (15/15)	100% (11/11)	100% (11/11)
SVR4/8, % (n/m[a])	39% (12/31)	87% (26/30)	80% (16/20)	89% (17/19)	100% (15/15)	100% (10/10)	90% (9/10)
Breakthrough, n	0	0	0	0	0	0	0
Relapse, n	19	4	4	2	0	0	1

G1 presents SVR8 data; G3 presents SVR4 data.
[a] Excludes 3 patients with nonvirologic discontinuation: G1 cirrhotic 8-week arm (2 patients); G3 cirrhotic (1 patient).

Results: A total of 143 patients were enrolled; 66% male, 98% white, and 45% Hispanic. Of the 102 G1 patients, 82% were G1a. Mean viral load at baseline was 6.65 $\log_{10}$ IU/mL. The study is currently

ongoing; all patients have completed dosing (data are correct as of 11/21/2014). The combination was safe and well tolerated. No patient discontinued because of a drug-related adverse event and no drug-related SAE occurred.

Conclusions: This novel study uses 3 potent, once-daily DAAs, GZR/EBR FDC plus SOF, combined in a pan-genotypic regimen to assess treatment durations of 4 to 12 weeks in cirrhotic and noncirrhotic patients with G1 and G3 HCV infection. This study will expand the understanding of treatment duration and help determine how to minimize drug exposure while maximizing SVR rates.

O007

ALL ORAL HCV THERAPY IS SAFE AND EFFECTIVE IN PATIENTS WITH DECOMPENSATED CIRRHOSIS: INTERIM REPORT FROM THE HCV-TARGET REAL WORLD EXPERIENCE

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Background and Aims: Interferon-based therapy of chronic hepatitis C (HCV) is ineffective and poorly tolerated in decompensated cirrhosis. All oral, direct-acting antiviral regimens are expected to be better tolerated and achieve higher rates of sustained virologic response (SVR). We evaluated the safety and efficacy of all oral HCV therapy in patients with decompensated cirrhosis participating in an ongoing multi-center study: HCV-TARGET.

Methods: Clinical, adverse events, and virologic data were collected throughout treatment and post-treatment follow-up between December 2013 and November 2014. The analyses were restricted to patients with cirrhosis and MELD score of ≥10 who had not undergone liver transplantation. The Safety Cohort consisted of patients who reached an end-treatment (EOT) time point while the Efficacy Cohort had a known virologic outcome (treatment failure or SVR4).

Results: To date, 277 patients with a diagnosis of cirrhosis and a MELD ≥10 were started on all oral regimen for HCV treatment; 217 were part of the Safety Cohort, and 156 reached post-treatment follow-up (Efficacy Cohort). Mean age was 59 yrs, 69% male, with MELD score range from 10 to 28 (mean 13.0). 58.5% had failed prior antiviral therapy and 9.7% had failed prior telaprevir or boceprevir triple therapy. Total bilirubin ranged from 0.1–15 (mean 2.1), albumin 1.4–5.0 (mean 3.2), platelets 19K-567K (mean 83K), creatinine 0.4–6 (mean 0.9). Genotype distribution and treatment regimens are shown in table. At least one AE was reported by 88% of all patients, although most were mild. For patients with available pre/post-treatment bilirubin values, 46/58 (80%) improved, 2/58 (3%) were unchanged, while 10/58 (17%) worsened. Similarly, among patients with pre/post-treatment albumin values, 33/54 (61%) increased, 7/54 (13%) unchanged, while 14/54 (26%) decreased. 26 patients had baseline MELD and post treatment week 4 data available; 18 had improvement, 5 unchanged, and 3 worsened. SVR 4 rates in those with available data are shown in the table.

Conclusions: All oral therapy with SOF/SMV ± RBV or SOF + RBV is well tolerated and efficacious in patients with decompensated cirrhosis. Importantly, markers of hepatic and synthetic function improved during the short-term follow-up.

Total cohort	SOF / RBV n = 120	SOF/SMV n = 123	SOF/SMV/RBV n = 34	All n = 277
Genotype, n (%)				
GT 1	45 (38%)	122 (99%)	32 (94%)	199 (71.8%)
GT 2	35 (29%)	0 (0%)	0 (0%)	35 (12.6%)
G3	37 (31%)	0 (0%)	0 (0%)	37 (13.4%)
G4	2 (2%)	0 (0%)	2 (6%)	4 (1.4%)
Other	1 (1%)	1 (1%)	0 (0%)	2 (1%)
MELD				
10–15	97 (81%)	104 (84%)	29 (85%)	230 (83%)
16–21	20 (17%)	12 (10%)	4 (12%)	36 (13%)
>21	3 (2%)	7 (6%)	1 (3%)	11 (4%)
Discontinued due to				
AE	3 (3%)	5 (4%)	3 (9%)	11 (4%)
Lack of efficacy	3 (3%)	0 (0%)	0 (0%)	3 (1.1%)
Died	0 (0%)	2 (2%) (liver failure, vascular shock)	1 (3%) (cause unknown)	3 (1.1%)
Virological response	(G2 only)	(G1 only)	(G1 only)	
SVR 4	18/24 (75%)	55/71 (77%)	13/16 (81%)	
Relapse	3/24 (12%)	15/71 (21%)	3/16 (19%)	
Breakthrough	1/24 (4%)	0/71 (0%)	0/16 (0%)	
Non-response	1/24 (4%)	1/71 (1%)	0/16 (0%)	
Loss to F-up	1/24 (4%)	0/71 (0%)	0/16 (0%)	

O008

EFFICACY AND SAFETY OF GRAZOPREVIR AND ELBASVIR IN HEPATITIS C GENOTYPE 1-INFECTED PATIENTS WITH CHILD–PUGH CLASS B CIRRHOSIS (C-SALT PART A)

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Background and Aims: Improved treatments are needed for patients with hepatitis C virus (HCV) infection and advanced cirrhosis. In the C-WORTHY study, the combination of grazoprevir (MK-5172, GZR; HCV NS3/4A inhibitor) and elbasvir (MK-8742, EBR; NS5A inhibitor) showed high rates of efficacy in patients with HCV infection with and without compensated cirrhosis. The purpose of the phase 2/3 C-SALT study (PN059) is to assess the efficacy, safety and pharmacokinetics (PK) of GZR plus EBR in patients with HCV G1, G4 or G6 infection and Child–Pugh (CP) class B cirrhosis. The phase 2 portion of C-SALT (part A) is described here.

Methods: CP-B patients with HCV infection received GZR (50 mg QD) and EBR (50 mg QD) for 12 weeks without RBV. Noncirrhotic patients with HCV infection were enrolled for PK analyses and received GZR (100 mg QD) plus EBR (50 mg QD) for 12 weeks. Plasma samples for PK analyses were collected in a subset of CP-B and noncirrhotic patients over 24 hours at TW4. The primary end point is SVR12 in CP-B patients (HCV RNA <LLoQ [15 IU/mL]).

Results: 30 CP-B and 10 noncirrhotic patients were enrolled. Among the CP-B patients, 57% were male, 97% were white, 100% had G1 infection (G1a, 87%), mean MELD was 9.79 (SD, 2.46), mean albumin was 3.4 g/dL (range, 2.6–4.8) and mean platelet count was $83 \times 10^3/\mu L$ (range, 36–175). The study is ongoing; 25 CP-B patients have reached EOT (table). No patient had virologic breakthrough, had a treatment-related SAE, discontinued study drug, or had a grade 3 or 4 ALT elevation. Four patients had transient grade 3 total bilirubin elevations without increased ALT or AST. One patient developed spontaneous bacterial peritonitis and died because of hepatic failure after EOT. In CP-B (n = 9) and noncirrhotic (n = 9)

patients, steady-state preliminary plasma C2 h, C24 h and AUC0–24 concentrations were 1.12 (0.62, 2.00), 1.73 (0.87, 3.43), and 1.30 (0.76, 2.21) for GZR, and 0.95 (0.70, 1.30), 1.07 (0.75, 1.53), and 0.93 (0.69, 1.25) for EBR, respectively.

Table: Patients with HCV RNA <15 IU/mL

Population	TW4	TW8	EOT	FW4
CP-B (n = 30)	76.7% (23/30)	96.4% (27/28)	100% (25/25)	100% (13/13)

CP-B, Child–Pugh Class B; EOT, end of treatment; FW, follow-up week; TW, treatment week.

Conclusions: High rates of virologic response were observed in CP-B patients receiving a combination of GZR plus EBR. The regimen was well tolerated with no evidence of significant hepatotoxicity. Plasma GZR exposure was slightly higher in CP-B patients receiving 50 mg compared with noncirrhotic patients receiving 100 mg; EBR exposure was similar in both groups. Full efficacy (including EOT and SVR12) and safety results will be presented.

General viral hepatitis

O009

A PROSPECTIVE STUDY OF HEPATITIS B REACTIVATION IN PATIENTS WITH PRIOR HBV EXPOSURE UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION: REACTIVATION ASSOCIATION WITH GRAFT-VERSUS-HOST DISEASE

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Background and Aims: Patterns of hepatitis B virus (HBV) reactivation in hepatitis B surface antigen (HBsAg)-negative, antibody to the hepatitis B core antigen (anti-HBc)-positive individuals undergoing hematopoietic stem cell transplantation (HSCT) have not been well described.
Methods: From September 2011 onwards, we recruited treatment-naïve HBsAg-negative, anti-HBc-positive patients with baseline undetectable serum HBV DNA (<10 IU/mL), undergoing either autologous or allogenic HSCT. For allogenic HSCT, only recipients whose donors were HBsAg-negative were recruited. Liver biochemistry, serum HBV DNA, HBsAg and antibody to HBsAg (anti-HBs) were prospectively monitoring every 4 weeks after HSCT up to 2 years. Following guidelines from the European Association for the Study of the Liver, entecavir was started when HBV reactivation, defined as detectable HBV DNA (≥10 IU/mL) was encountered.
Results: At the time of writing, among 328 patients undergoing HSCT, 89 (27.3%) were HBsAg-negative, anti-HBc-positive, of whom 67 (75.3%) fulfilled our inclusion criteria and were recruited. The median duration of follow-up was 52 (range 4–104) weeks. The 2-year cumulative HBV reactivation rate, calculated using the Kaplan–Meier method, was 36.8%. Thirteen patients developed reactivation after a median duration of 44 (range 8–100) weeks, of whom 11 patients (84.6%) remained HBsAg-negative at reactivation. Median HBV DNA level at reactivation was 24.8 (range 14.6–428) IU/mL. Patients with acute and/or chronic graft-versus-host-disease (GVHD) had a significantly higher 2-year cumulative rate of HBV reactivation than those without (acute GVHD: 78.8% *versus* 22.4%, p = 0.006; chronic GVHD: 83.8% *versus* 23.3%, p < 0.001). Other clinical parameters, including age, anti-HBs status, and donor serology had no association with HBV reactivation (all p > 0.05). Multivariate Cox regression analysis showed that chronic GVHD was the only factor independently associated with reactivation (p = 0.030, hazard ratio 3.9, 95% confidence interval 1.1–13.8). Entecavir treatment successfully controlled HBV reactivation in all cases.
Conclusions: From this interim analysis, a substantial proportion of HBsAg-negative, anti-HBc-positive patients developed HBV reactivation after HSCT, with risk of reactivation significantly higher in patients with chronic GVHD. Periodic HBV DNA monitoring was an effective strategy in preventing HBV-related complications.
ClinicalTrials.gov identifier NCT01481649
Supported by the Health and Medical Research Fund, Hong Kong

O010

T AND B CELL RESPONSES AND PREVIOUS EXPOSURE TO HEPATITIS B VIRUS IN "ANTI-HBC ALONE" PATIENTS

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Background and Aims: A serologic response to hepatitis B virus (HBV), defined as "anti-HBc alone" is commonly observed, but its significance remains unclear. This study aimed to define the relationship between "anti-HBc alone" serostatus and HBV infection, including HBV-specific T and B cell memory responses.
Methods: 31 "anti-HBc alone" patients were enrolled. Total HBV DNA and cccDNA were tested by nested polymerase chain reaction (PCR) analysis in liver samples from 22 "anti-HBc alone" patients *vs* controls (chronic or resolved HBV infection), followed by HBsAg/HBcAg immunohistochemical (IHC) staining. IFN-γ secretion by HBV-specific T cells was compared in individuals who were "anti-HBc alone" (n = 27), resolved HBV (n = 21), chronic HBV (n = 24) and 12 healthy controls using enzyme-linked immunospot (ELISpot) assays. An HBsAg-IgG B-cell ELISpot assay was performed in "anti-HBc alone" patients before and after a booster dose of recombinant HBsAg vaccine.

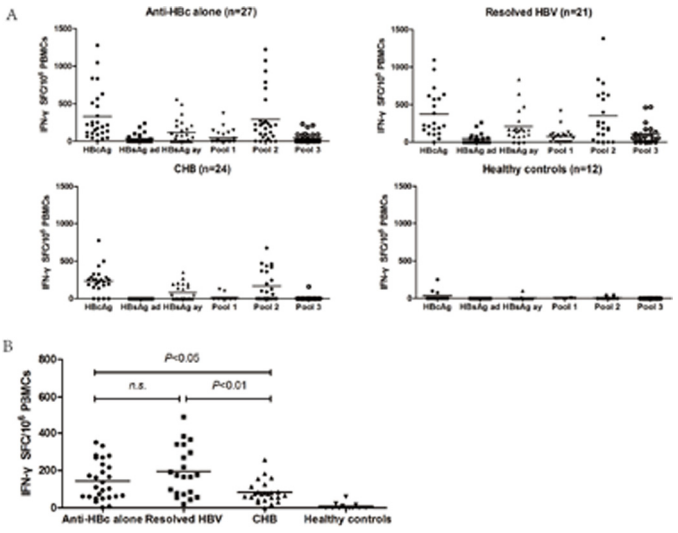

Figure 1. Analysis of HBV-specific IFN-γ producing T cells performed by ELISpot after stimulation with different HBV proteins and peptides, respectively. (A) Pooled ELISpot data for all groups. (B) Comparison of mean spots of the positive tests and frequency of positive responses (calculated on all HBV recombinant protein antigens and peptide pools) obtained in four groups are presented.

Results: Infrequent intrahepatic total HBV DNA (2/22, 9.1%) and cccDNA (1/22, 4.5%) were detected in biopsies; HBsAg and HBcAg IHC staining was negative. HBV-specific T cell responses were similar between "anti-HBc alone" individuals and HBV resolvers (Figure 1). Circulating HBV-memory B cell responses were detected in all "anti-HBc alone" individuals, consistent with an HBsAg-specific memory pool. After one HBV vaccine dose, increased anti-HBs antibody levels were observed, accompanied by an expansion of HBsAg-specific memory B cells ($P = 0.0226$) (Figure 2).
Conclusions: "Anti-HBc alone" individuals showed HBV-specific T cell and memory B cell responses typical of previous viral exposure and protective memory, suggesting a resolved infection.

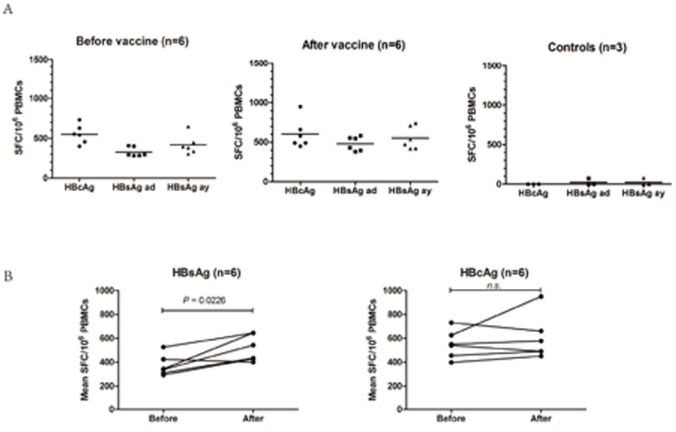

Figure 2. Analysis of HBV-specific memory B cell ELISpot responses. (A) HBV-specific B cell responses shown in SFC/106PBPMC before and after one dose of HBV vaccinated "anti-HBc alone" patients compared with three negative controls (without HBV vaccination or exposure to HBV infection). (B) Comparison of memory B cell responses against HBsAg and HBcAg protein antigen in six "anti-HBc alone" individuals before and after one dose of HBV vaccine.

O011
A NATIONWIDE SURVEY OF HEPATITIS E VIRUS INFECTION IN LIVER TRANSPLANT RECIPIENTS IN JAPAN

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Background and Aims: Hepatitis E virus (HEV) infection induces self-limiting liver disease in immunocompetent individuals. Recently, chronic hepatitis E has been reported in several immunosuppressed patients after organ transplantation. However, the prevalence of HEV infection after liver transplantation is still unknown in Japan. In the present study, we carried out a nationwide survey of the prevalence of hepatitis E virus infection in liver transplant recipients in Japan.
Methods: 1830 patients who received liver transplants and presently alive at the19 university hospitals in Japan were included in the present study. Serum samples of all patients were examined for the anti-HEV immunoglobulin G (IgG), anti-HEV immunoglobulin M (IgM), and anti-HEV immunoglobulin A (IgA) by anti-HEV enzyme-linked immunosorbent assay (ELISA). Also, HEV RNA in serum samples was analyzed with real time polymerase chain reaction (PCR).
Results: All patients were investigated for HEV infection. The prevalence of anti-HEV IgG was 2.6% (48/1830), anti-HEV IgM was 0.05% (1/1830), and anti-HEV IgA was 0% (0/1830). 1574 patients were also tested for HEV RNA in serum. Two patients were found positive for HEV RNA (0.13%; 2/1574). One of the two patients developed chronic HEV hepatitis after liver transplantation. In addition, HEV RNA was detected in one of the transfused blood units. Complete matching of HEV sequences between donor and recipient was observed in this case. The patient was infected with HEV via transfused blood from a volunteer donor. After Ribavirin therapy at a 600 mg per day for 1 month, the serum HEV RNA level decreased to an undetectable level, and the patient cleared infection under the treatment.
Conclusions: We conclude that the prevalence of HEV infection in liver transplant recipients is very low in Japan; however, liver transplant recipients have a risk for chronic HEV infection. Also, a potential risk of posttransfusion hepatitis E should be considered.
This work was supported by a Health Labour Sciences Research Grant (2012)

O012
HEPATITIS DELTA VIRUS CAN SURVIVE LIVER REGENERATION AND IS AMPLIFIED THROUGH HUMAN CELL DIVISION BOTH IN VITRO AND IN VIVO

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Background and Aims: Intrahepatic latency of the Delta antigen (HDAg) has been detected even months after liver transplantation and we previously showed that a latent HDV mono-infection can persist in vivo for at least 6 weeks in the absence of HBV before being converted into a productive HBV/HDV infection (Giersch, J. Hepatology 2014). Although these studies highlight the ability of HDV to persist in quiescent hepatocytes, little is known about the impact of cell proliferation on HDV persistence. Aim of the study was to evaluate the ability of HDV to survive cell division in vitro using NTCP-transduced hepatoma cell lines and in vivo using HBV/HDV infected human liver-chimeric uPA/SCID mice.
Methods: NTCP-transduced HepG2 cells were infected with culture-derived HDV or HBV/HDV serum (both MOI=1), treated from day 2 post infection with the entry inhibitor Myrcludex-B, passaged every 7 days (each time 1:3 dilution) for a period of 42 days. In vivo cell proliferation was triggered by transplanting primary human hepatocytes (PHHs) isolated from HBV/HDV infected humanized mice into naïve recipients. Virological parameters were determined by qRT-PCR. Presence of HDAg, HBcAg and cell proliferation (Ki67), were determined by immunofluorescence.
Results: Despite 6 in vitro cell passages (1:243 final cell dilution) and Myrcludex-B-mediated block of extracellular viral spreading, comparable amounts of HDAg-positive cells (median 4–6%) and HDV RNA expression levels (median 4×10^3 HDV-RNA/GAPDH) were detected after each cell harvesting. Notably, HDAg-positive cells in passaged cultures appeared in clusters and Ki67-HDAg co-staining indicated that infected cells proliferated as often as uninfected cells. Comparable results were seen also using a culture-derived (HBV-free) HDV inoculum. In vivo expansion of PHHs isolated from HBV/HDV-infected humanized mice was confirmed 2, 4 and 8 weeks after transplantation. While intrahepatic and serological HBV markers rapidly dropped in proliferating PHHs, HDAg-positive PHHs were observed at all-time points, preferentially forming clusters of adjacent HDAg-positive PHHs at week 4 and 8 post transplantation, indicating maintenance of HDV infection among dividing PHHs and despite the absence of detectable HDV viremia.

Conclusions: Our in vitro and in vivo findings suggest that by surviving hepatocyte proliferation, HDV may not only persist but even be able to propagate, at least to some extent, among daughter cells, despite the absence of HBV.

O013

EVALUATION OF HDV QUANTIFICATION ASSAYS WORLDWIDE: RESULTS OF THE FIRST HDV INTERNATIONAL QUALITY CONTROL STUDY

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Background and Aims: Hepatitis Delta virus (HDV), a satellite of hepatitis B virus is responsible for a more severe liver disease. Monitoring of infected-patients is based on quantification of the plasma RNA viral load (VL) using real time RT-PCR assays. Commercial kits were recently shown to significantly underestimated or failed to quantify HDV-VL. The first international quality control was organized to evaluate almost all HDV quantification assays mostly "home-made" by also commercials assays used routinely. The newly developed WHO HDV international standard (WHO-IS) allowed relevant comparison studies between the different assays.
Methods: Twenty clinical samples with various VL loads and genotypes, including 2 negative controls and a second panel of 8 samples composed of 2 dilutions (1:10 and 1:100) of the WHO-IS in triplicate together with 2 negative controls, were sent blindly for quantification to 27 laboratories worldwide: Australia (1), Belgium (1), Denmark (1), France (6), Greece (2), Germany (4), Italy (3), Luxembourg (1), Mauritania (1), Spain (1), Russia (1), Switzerland (1), Turkey (1), United Kingdom (2) and the USA (1).
Results: Twenty-four labs sent their results for the 2 panels of samples. Results obtained with the WHO-IS panel, showed a very good repeatability while 3 labs found positive 1 or the 2 negative controls of this panel. Considering the results converted into IU/mL of these 24 labs for the 20 clinical samples, the 2 negative controls were found undetectable; 12 labs (50%) failed to detect 1 or several samples with expected VL values ranging from 3.9 to 6.8 IU/mL and 7 (29.2%) underestimated (>1 to 3 log) most samples, whatever the genotype of the infected-strain. Four laboratories found comparable results whatever the nucleic acid extraction method or RT-PCR technology or devices used. Primers and probes were designed within the ribozymes regions of the genome for 3 of them and within the Delta antigen-coding region for the remaining one. Of note this latter assay underestimated 2 samples of genotype 5 and 8 by 3 and 1 log.
Conclusions: The current assays for quantification of HDV RNA seem to date very imperfect for the diagnosis and monitoring of patients. The main points toward standardization of quantification assays should consider HDV genetic variability; an internal control for all steps; automation and the use of the available WHO-IS.

O014

A TARGETED RNAi SCREEN USING A HIGH-THROUGHPUT INFECTIOUS MODEL SYSTEM UNCOVERS GLYPICAN GPC5 AS A HOST FACTOR FOR HEPATITIS B AND D VIRUS ENTRY

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Background and Aims: Chronic hepatitis B virus (HBV) infection and its co-infection with hepatitis D virus (HDV) are leading causes of liver disease and cancer world-wide. Viral entry is the first step of infection, plays a key role in spread and control of infection and has been shown to be a viable target for the development of curative therapeutic strategies. HBV and HDV viruses infect exclusively hepatocytes and share the same envelope proteins and entry pathway. Heparan sulfate proteoglycans (HSPGs) have been shown to mediate HBV and HDV attachment at the hepatocyte cell surface before interacting with sodium taurocholate cotransporting polypeptide (NTCP). However, the detailed mechanisms of entry and its host cell-dependency factors are still poorly understood. Using a targeted RNAi screen we aimed to investigate the role of HSPG core proteins in HBV/HDV entry.
Methods: We established a high-throughput HDV infection model using Huh7 cells overexpressing NTCP and susceptible to HDV infection. Unlike previous approaches, this system does not require dimethylsulfoxide or polyethylene glycol to facilitate entry, thus providing a model closely mimicking natural infection. Using a small library of siRNAs targeting the core members of the HSPG family, we performed a targeted screen to evaluate the role of individual HSPG core proteins in HDV entry.
Results: While the silencing of the expression of most HSPG core genes did not result in strong modulation of infection, silencing of Glypican GPC5 expression induced a marked and significant decrease of HDV infection as shown by immunofluorescence of HDV infected Huh7-NTCP+ cells and RT-PCR of HDV RNA. Using HepG2 cells overexpressing NTCP and individual siRNAs, we demonstrate that GPC5 silencing is a host cell-dependency factor for HBV infection. Silencing of GPC5 in HepG2-NTCP cells resulted in a marked decrease of both HBV-positive cells and HBV pregenomic RNA, confirming the functional role of this host cell surface protein for both HDV and HBV infection.
Conclusions: Collectively, this targeted RNAi screen in a high-throughput infectious model system uncovers GPC5 as an entry factor for hepatitis B and D viruses. These results advance our understanding of cell entry of HBV and HDV and open new avenues for therapies aiming at HBV cure. Since glypicans have been shown to play a role in the control of cell division and growth regulation, virus-GPC5 interactions may also play a role for pathogenesis of virus-induced liver disease.

O015

QUANTITATIVE MEASUREMENTS OF MUTATIONS IN BASAL CORE PROMOTOR REGION SUGGEST A LOSS OF IMMUNE TOLERANCE IN HBeAg POSITIVE CHRONIC HEPATITIS B INFECTIONS

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Background and Aims: The standard clinical definition of immune tolerant (IT) CHB includes HBeAg positivity, high HBV DNA, normal ALT and minimal hepatic necroinflammation, assuming aBVHBV predominantly wildtype HBV quasispecies under no immune pressure. The GS-US-203-0101 trial evaluated TDF vs TDF+FTC therapy in IT patients, and we previously identified the presence of immune evasion mutations in a subset these patients using population sequencing, suggesting that current clinical criteria need updating. The aim of this study was to quantify immune evasion mutations by Next Generation Sequencing (NGS) and evaluate their impact at baseline and on-treatment.
Methods: Whole genome NGS was performed on baseline samples (Illumina Miseq). A bioinformatics pipeline assembled validated and mapped the sequencing data. This was analysed against baseline demographic, clinical, and virological data, as well as on-treatment outcomes after 192 weeks.
Results: Data were available for 99 patients (median age 32 yrs, 51% male, 95% Asian, genotype B/C 58%/42%; median ALT 25 IU/mL, HBV DNA 8.5 $\log_{10}$ IU/mL, HBsAg 4.8 $\log_{10}$ IU/mL, HBeAg 3.6 $\log_{10}$PEIU/mL). NGS detected and quantified mutations present >1%, including those at low frequency (<20%) that were not seen by population sequencing (see Table 1).
The levels of BCP mutations fell into three categories, <1%, 1–5% and >45%. Linear regression showed higher prevalence was associated with older age ($p = 0.001$) and lower baseline HBsAg and HBeAg titres ($p < 0.001$). A threshold of >1% BCP mutations at baseline was analysed with respect to treatment outcomes alongside previously identified parameters including treatment arm, baseline serology and genotype. >1% BCP was independently associated with improved viral suppression (89% vs 58%, OR = 12, $p = 0.005$) and increased HBeAg decline (OR = 27, $p = 0.004$). Clinically relevant thresholds for other mutations were investigated; core P130T >1% negatively impacted on HBeAg decline (OR = 0.16, $p = 0.02$) and NRE G1613A >5% negatively impacted viral suppression (OR = 0.24, $p = 0.02$).

Table 1. Key mutations

	NGS	Population sequencing
Negative Regulatory Element (NRE) G1613A	73 (74%)	19 (19%)
Basal Core Promotor (BCP) 1762/1764	23 (23%)	12 (12%)
BCP T/C1858G	0 (0%)	0 (0%)
Precore (PC) G1896A	15 (15%)	3 (3%)
Core P130T	47 (47%)	6 (6%)
PreS1 M1	33 (33%)	4 (4%)
PreS2 M1	11 (11%)	6 (6%)
HBsAg P120T/G145R	9 (9%)	4 (4%)
RT 80/169/173/180/181/184/202/204/236/250	12 (12%)	0 (0%)

Conclusions: BCP mutations are a virological response to host immune pressure. An increased prevalence was associated with lower HBeAg and HBsAg titres, improved viral suppression and HBeAg decline on-treatment. These findings demonstrate baseline heterogeneity within this immune tolerant population possibly due to variability in immune pressure. Furthermore, these results suggest that the current definition of IT may need to be updated to include detection of BCP variants.

O016

HEPATITIS B SURFACE ANTIGEN AND DNA LEVELS CAN IDENTIFY INACTIVE CARRIERS AND PREDICT LOWER RISK FOR HEPATOCELLULAR CARCINOMA AND CIRRHOSIS AMONG GENOTYPE B AND C CHRONIC HEPATITIS B CARRIERS

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Background and Aims: Inactive chronic hepatitis B virus (HBV) carriers have significantly decreased risk for hepatocellular carcinoma (HCC) and liver cirrhosis, and are more likely to reach HBsAg seroclearance. Differentiation between inactive and active carriers is crucial to identifying those in need of more stringent follow-up. Previous studies showed that a single point measurement of HBV DNA and HBsAg levels could identify inactive carriers in genotype D carriers. This study aims to validate these previous findings in genotype B and C carriers, and also examines rates of HCC, cirrhosis, and HBsAg seroclearance among those identified as inactive carriers using a single measurement.
Methods: 1526 HBeAg-negative individuals from the REVEAL-HBV cohort were included in this study. Participants were anti-HCV seronegative and free of cirrhosis at study entry. To identify inactive and active carriers, all participants had at least two to three measurements of HBV DNA / ALT within 1.5 years of study entry. Hazard and rate ratios of outcomes were estimated using Cox proportional hazards models.
Results: Within 1.5 years, 775 (51%) were inactive carriers (IC) with HBV DNA persistently <10,000 copies/mL, 496 (33%) were active carriers (AC) with periodic HBV DNA elevations <100,000 copies/mL, and 255 (17%) were highly replicative active carriers (HRAC) with HBV DNA fluctuating or persisting above 100,000 copies/mL. The proportion of abnormal ALT elevations (>40 U/L) was 0%, 9%, and 20% for IC, AC, and HRAC, respectively. A single point measurement of HBsAg <1000 iu/mL and HBV DNA <10,000 copies/ml could identify IC with a sensitivity, specificity, positive predictive value, and negative predictive value of 0.71, 0.85, 0.83, 0.74, respectively, and 0.71, 0.91, 0.96, 0.51, respectively, when limited to IC and HRAC only. The same single point measurement also identified individuals with significantly lower risk of HCC and cirrhosis, with adjusted hazard ratios (95% CI) of 0.20 (0.08–0.51) and 0.27 (0.14–0.50), respectively. Rates of HBsAg seroclearance were also much higher, with an adjusted rate ratio (95% CI) of 7.14 (5.62–9.09).
Conclusions: Compared with long-term determination, a single point combined measurement of HBsAg and HBV DNA is able to identify inactive carriers with moderate accuracy, while also differentiating those with reduced risk for HCC and cirrhosis, and higher probability of HBsAg seroclearance.

Non-invasive marker of liver disease management

O017

EFFECTIVENESS OF TREATMENT INITIATION BASED ON FIBROSIS STAGE ASSESSED BY METHODS OF FIBROSIS DIAGNOSIS IN TERMS OF 5-YEAR INCIDENCE OF MORBI-MORTALITY

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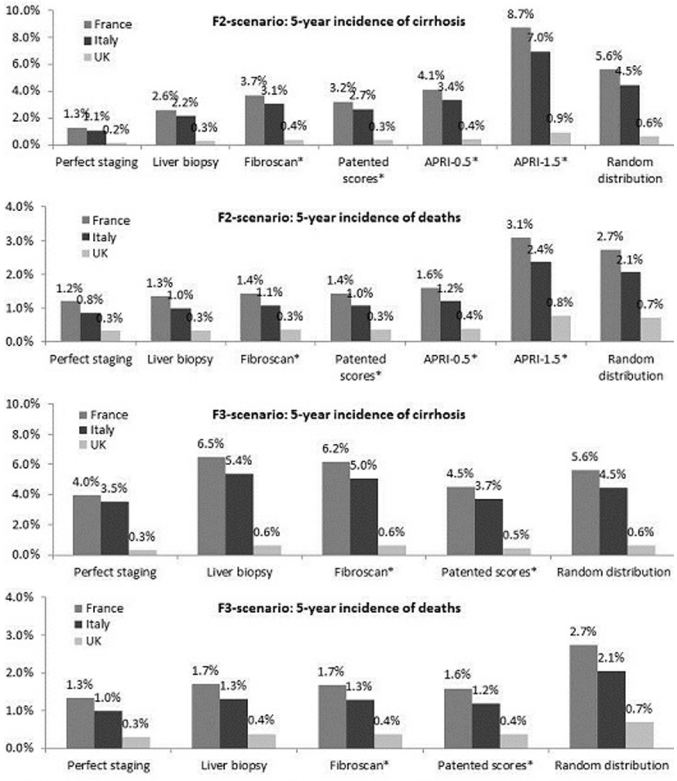
Background and Aims: European and American experts recommend targeting HCV antiviral therapy to patients (pts) with fibrosis ≥F2, with the highest priority assigned for F3–F4. The impact of targeted therapy has never been evaluated in light of the risk of misclassification of the methods of fibrosis diagnosis.
Methods: A country-specific decision model was used to predict 5-year (yr) incidence of cirrhosis and deaths in three countries with different profiles of natural history (France, Italy, UK). Fibrosis misclassification was integrated for different methods: liver biopsy (LB), fibroscan (FS), patented scores (fibrotest, hepascore, fibrometer), and APRI. IFN-free regimens were initiated considering two scenarios where pts are distributed in fibrosis stage ≥F2 (F2-scenario) and ≥F3 (F3-scenario), according to available thresholds of methods. Outcomes based on the use of these methods were compared to those using a perfect staging (no misclassification) and a random distribution (50% of pts were considered for therapy regardless of fibrosis stage).
Results: In F2-scenario, 5-yr incidence of cirrhosis is: 0.2–1.3% using the perfect staging, 0.3–2.6% with LB, 0.4–3.7% with FS, 0.3–3.2% with patented scores, 0.4–4.1% with APRI-0.5, 0.9–8.7% with APRI-1.5, 0.6–5.6% with random distribution; 5-yr incidence of deaths is: 0.3–1.2% using the perfect staging, 0.3–1.3% with LB, 0.3–1.4% with FS, 0.3–1.4% with patented score, 0.4–1.6% with APRI-0.5, 0.8–3.1% with APRI-1.5, 0.7–2.7% with random distribution. In F3-scenario, using the perfect staging, 5-yr incidence of cirrhosis increases dramatically, at least for France and Italy, whereas this scenario has less impact on 5-yr incidence of liver deaths; using methods of diagnosis and due to their risk of misclassification, 5-yr incidence of cirrhosis shows 28–60%> relative increase compared to F2-scenario in France and Italy, whereas the increase is lower in UK, as well as 5-yr incidence of liver deaths.
Conclusions: Based on the unrealistic scenario of perfect staging, F2- and F3-scenarios are efficient to reduce 5-yr incidence of liver-related deaths but F3-scenario is not optimal in terms of 5-yr incidence of cirrhosis. LB, FS and patented scores are efficient to select subgroups with higher risks of cirrhosis and mortality: F2- and F3-scenarios are still efficient in terms of 5-yr incidence of mortality although their impact would be lower than perfect staging; conversely, they are associated with deleterious impact on 5-yr incidence of cirrhosis, mainly F3-scenario.



*Thresholds considered were: F2-scenario: 7.0 for FibroScan, 0.49 for FibroTest, 0.5 for Hepascore, 0.411 for Fibrometer, and 0.5 (APRI-0.5) and 1.5 (APRI-1.5) for APRI; F3-scenario: 9.5 for FibroScan, 0.59 for FibroTest, 0.5 for Hepascore, 0.628 for Fibrometer (no available thresholds for Hepascore and APRI).

O017A

NON-INVASIVE TOOLS AND RISK OF VARICES AND CLINICALLY SIGNIFICANT PORTAL HYPERTENSION IN COMPENSATED CIRRHOSIS: THE "ANTICIPATE" STUDY

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Background and Aims: Current guidelines recommend endoscopic screening for varices in all patients with cirrhosis. Several non-invasive markers have been assessed but none have permeated clinical practice. This might be, in part, due to the use of performance measurements without direct clinical translation (sensitivity, specificity, ROC curves). Risk prediction modeling could be a better way to answer relevant clinical questions, such as what would be the risk of varices (V), varices needing treatment (VNT: medium-large varices or small with red signs) and clinically significant portal hypertension [CSPH: hepatic venous pressure gradient (HVPG) ≥10 mmHg] given a certain value of these non-invasive tests. The aim of this study was to construct risk prediction models based non-invasive tests to determine the risk of V, VNT and CSPH in patients with compensated Child A cirrhosis.
Methods: We analysed data from Child A patients with cirrhosis from 5 centers in Europe and Canada with paired endoscopic (n = 542) or HVPG (n = 315) and non-invasive data: Fibroscan (n = 542), LSPS (n = 299) and Platelet-spleen ratio (PSR, n = 299). Risk of V, VNT and CSPH was modeled with logistic regression (bootstrapped). Examples of potential decision thresholds are provided.
Results: Prevalence of V was 42% and of VNT 13%. LSPS-based model had the best accuracy in predicting V (AUC:0.79). LSPS of 0.7 was associated with a risk of V of 10% (Figure 1). However, only 6% of the patients had a LSPS <0.7. Fibroscan and PSR models still had acceptable accuracy, but could not identify a subgroup with

<10% risk of V. LSPS was also the best predictor for VNT (AUC:0.79). LSPS of 1.36 and 2.38 were associated with a 5 and 10% risk of VNT, and 24% and 51% of the sample had values below these thresholds. PSR also had good accuracy (AUC:0.74), with values of 1700 and 1050 predicting a risk of VNT of 5 and 10%. Fibroscan had a lower accuracy that LSPS and PSR (AUC:0.68). Values of 13.5 and 23 Kpa were associated with a 5 and 10% risk of VNT. Prevalence of CSPH was 63%. LSPS-based model had the best accuracy in predicting CSPH (AUC: 0.88). LSPS values of 0.71, 1.66 and 2.63 were associated with risks of CSPH of 20, 50 and 80% respectively (Figure 2). Only 6% of the patients had a predicted risk of CSPH <20%, and none of <10%. Fibroscan had also an excellent accuracy (AUC: 0.82), but could not identify a subpopulation with a very low risk of CSPH. Fibroscan values of 18 and 28 were associated with a risk of 50 and 80% of CSPH. Accuracy of PSR was lower. The best data-driven models did not outperform LSPS.

Conclusions: LSPS was the best of the 3 non-invasive markers to predict V, VNT and CSPH. In a population with a ~40% baseline risk of varices none of the tests were good enough to identify a subgroup with a <10% risk of varices. However, all three could identify a significant proportion of patients with a very low risk of VNT, suggesting that endoscopy could be avoided in 25–50% of compensated cirrhotic patients. LSPS and Fibroscan based models had an excellent performance at predicting the risk of CSPH and could reliably identify patients with a very high risk of CSPH. These tools, however, could not define a subgroup of patients with very low risk of CSPH.

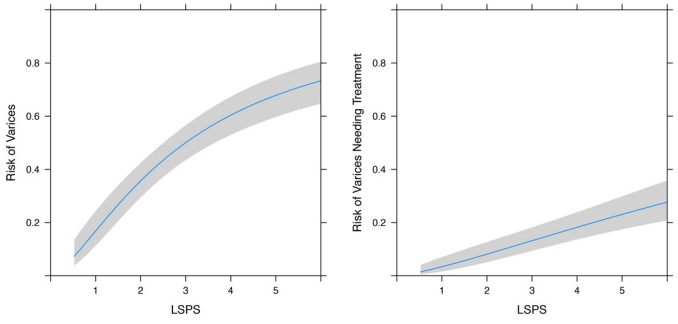

Figure 1.

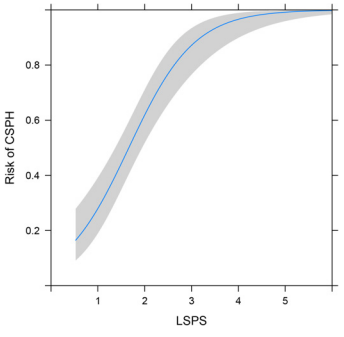

Figure 2.

O018

2D-SHEAR WAVE ELASTOGRAPHY IS EQUIVALENT OR SUPERIOR TO TRANSIENT ELASTOGRAPHY FOR LIVER FIBROSIS ASSESSMENT: RESULTS FROM AN INDIVIDUAL PATIENT DATA BASED META-ANALYSIS

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Background and Aims: 2D shear wave elastography (2D-SWE) based on supersonic shear imaging (SSI) has proven to be efficient for the evaluation of liver fibrosis in several small to moderate size trials. We aimed at running a larger scale analysis of individual data.

Methods: Clinical data from 1340 patients with 2D-SWE measurement and liver biopsy were collected retrospectively from 13 sites and analysed. Additionally, data on transient elastography (TE) were available in a subsample of 972 patients. The database was cleared by the French National Commission on Information Technology and Liberties and the study was registered on clinicaltrials.gov. The data were analysed using appropriate random effect models for ROC analysis as well as paired comparison of AUROC.

Results: Main etiologies were chronic hepatitis C (HCV, n = 470), hepatitis B (HBV, n = 420), non-alcoholic fatty liver disease (NAFLD, n = 172) or other liver diseases (n = 278). There was high heterogeneity between sites for liver disease etiologies and fibrosis stages. 40.8% of the patients had minimal or no fibrosis, 19.3% had significant fibrosis, 14.0% had severe fibrosis and 26.0% had cirrhosis.

Overall performance of 2D-SWE assessed by AUROC in patients with HCV, HBV and NAFLD was 86.3%, 91.6%, 85.9% for diagnosing significant fibrosis and 96.1%, 97.1% and 95.5% for diagnosing cirrhosis, respectively. Optimal cut-offs were 7.1 kPa for diagnosing significant fibrosis in all patients (75.7% correctly classified), 13.5 kPa for diagnosing cirrhosis in HCV and NAFLD patients, and 11.5 kPa for diagnosing cirrhosis in HBV patients (87% correctly classified).

Differences in AUROC were borderline significant for diagnosing significant fibrosis (95% CI for AUROC-2D-SWE minus AUROC-TE: [0.0004, 0.055], p = 0.047) and AUROC was significantly higher for 2D-SWE when diagnosing cirrhosis (95% CI for AUROC-2D-SWE minus AUROC-TE: [0.006, 0.036], p = 0.0058). 2D-SWE was superior for diagnosing cirrhosis specifically in HCV patients (AUROC difference: 0.018; p = 0.046) and HBV patients (AUROC difference: 0.062; p = 0.015). It was superior for diagnosing significant fibrosis in HBV patients (AUROC difference: 0.102; p = 0.0005) and severe fibrosis in NAFLD patients (AUROC difference: 0.152; p = 0.002).
Conclusions: 2D-SWE based on SSI showed good to excellent performance for non-invasive assessment of liver fibrosis, and was equivalent or superior to transient elastography.

O019
THE VALUE OF DIFFERENT NON-INVASIVE TESTS FOR PREDICTING THE PRESENCE OF CLINICALLY SIGNIFICANT PORTAL HYPERTENSION AND ESOPHAGEAL VARICES IN CIRRHOTIC PATIENTS

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Background and Aims: Diagnosis of clinically significant portal hypertension (CSPH) and esophageal varices (EV) in cirrhotic patients is crucial for timely initiation of prophylaxis of variceal bleeding but requires upper GI endoscopy or measurement of hepatic venous pressure gradient (HVPG). Our aim was to assess the diagnostic value of serological scores (initially developed for fibrosis evaluation) and transient elastrography (TE) for prediction of CSPH and EV.
Methods: Our study included 917 consecutive cirrhotic patients undergoing upper GI endoscopy, HVPG measurements, serological tests at the Medical University of Vienna between January/2004-January/2014. Simple serological scores were calculated: APRI, Fibrosis Index (FI), FIB-4, Forns score, King's and Lok score. In a large subset of 614 patients TE was performed at the same day of blood testing and HVPG measurement. According to the newly proposed TE quality criteria, ten valid memeasurements with a median liver stiffness value ≥7.1 kPa and IQR/median >30% were considered poorly reliable and not included for analysis. The TE cases without 10 valid measurements were also excluded.
Results: CSPH was diagnosed in 70.4% of patients and 65.6% of those showed EV at upper GI endoscopy. Etiologies of liver cirrhosis were: viral (45.4%), alcoholic (35.2%), others (19.4%). Reliable LS measurements were obtained in 65.9% of patients. All serological scores and liver stiffness were significantly correlated with the presence of CSPH and EV (table). The highest AUCs for predicting both CSPH and EV were obtained for TE, Lok and FI score (table). The performance of all non-invasive tests for predicting EV was lower compared to that obtained for prediction of CSPH.

Test	Correlation with HVPG values and prediction of CSPH					Prediction of EV presence			
	Spearman correlation coefficient	Cut-off	AUC	Se (%)	Sp (%)	Cut-off	AUC	Se (%)	Sp (%)
APRI	r = 0.165, p < 0.0001	>2.22	0.665	43.2	86	>2.32	0.556	40.6	70
FI	r = 0.394, p < 0.0001	>3.29	0.834	66.4	90.1	>3.39	0.720	76.2	61.3
FIB-4	r = 0.325, p < 0.0001	>5.09	0.795	56.1	91.7	>5.19	0.671	69.4	59
Forns	r = 0.255, p < 0.0001	>8.32	0.770	65.8	82.5	>8.53	0.672	64.5	63.3
King's	r = 0.240, p < 0.0001	>38.8	0.731	58.4	80.2	>39.4	0.596	59.6	57.5
Lok	r = 0.472, p < 0.0001	>0.64	0.834	85.2	70.2	>0.74	0.743	80.2	60.8
TE	r = 0.650, p < 0.0001	>26.3	0.897	78.8	90.1	>33.3	0.751	69.3	70.1

Conclusions: TE demonstrates an excellent performance for predicting CSPH but has only moderate accuracy for predicting EVs. While TE failed or resulted in poorly reliable results in more than 30% of patients, serological socres such as the Lok or FI score can be easily calculated for all cirrhotic patients. Upper GI-endoscopy remains the gold-standard for surveillance for EV today

O020
MAGNETIC RESONANCE ELASTOGRAPHY IS SUPERIOR TO CLINICAL PREDICTION MODELS FOR DETERMINATION OF ADVANCED FIBROSIS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE: A PROSPECTIVE STUDY

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Background and Aims: Two-dimensional magnetic resonance elastography (2D-MRE), a novel magnetic resonance method for assessing liver stiffness, has been shown to have high diagnostic accuracy for predicting advanced fibrosis (stage 3 and 4 fibrosis) in patients with non-alcoholic fatty liver disease (NAFLD). However, head-to-head comparisons between 2D-MRE and clinical prediction rules using laboratory and demographic data have not yet been performed in a prospective cohort of patients with biopsy-proven NAFLD. The aim of this study was to compare the diagnostic accuracy of 2D-MRE with that of 8 previously defined clinical prediction rules (AST:ALT ratio, APRI, BARD, FIB-4, NAFLD Fibrosis Score, Bonacini cirrhosis discriminant score, Lok Index, and NASH CRN model) for predicting advanced fibrosis in a prospective study with paired liver biopsy as the gold standard.
Methods: This is a cross-sectional analysis of a prospective study of 102 patients (58.8% women) with biopsy-proven NAFLD. The patients underwent 2D-MRE within 1 year of biopsy and clinical and laboratory tests within 90 days of biopsy. ROC analysis was performed to assess the performance of MRE and clinical prediction rules in predicting advanced fibrosis. The radiologist was blinded to clinical data and the pathologist was blinded to pathology/imaging data. Biopsies were scored using the NASH-CRN histologic system.

	AUROC	95% CI	AUC comparison, MRE vs. clinical prediction models
2D-MRE	0.957	(0.918, 0.996)	N/A
Clinical prediction models			
AST to ALT ratio	0.825	(0.732, 0.918)	p = 0.013
APRI	0.807	(0.702, 0.911)	p = 0.006
BARD	0.816	(0.723, 0.910)	p = 0.001
FIB-4	0.861	(0.775, 0.946)	p = 0.039
NAFLD fibrosis score	0.818	(0.704, 0.932)	p = 0.013
Bonacini cirrhosis discriminant score	0.826	(0.725, 0.926)	p = 0.014
Lok index	0.838	(0.731, 0.944)	p = 0.046
NASH CRN model	0.796	(0.678, 0.915)	p = 0.009

CI, Confidence interval.

Results: The mean (± standard deviation) age and BMI were 51.3 (±14.0) years and 31.7 (±5.5) kg/m^2, respectively. The median (interquartile range) time interval between biopsy and MRE was 41 (48) days, and that between biopsy and collection of clinical predictor data was 29 (28) days. 48, 26, 9, 13, and 6 patients had stage 0, 1, 2, 3, and 4 fibrosis, respectively. The area under ROC curve (AUROC) was 0.957 for 2D-MRE and between 0.796 to 0.861 for the 8 clinical prediction rules. In head-to-head comparisons using

the Delong test, 2D-MRE had significantly better AUROC ($p < 0.05$) than each of the 8 clinical prediction rules for predicting advanced fibrosis, as shown in the table.

Conclusions: Compared to clinical prediction rules, 2D-MRE provides significantly higher accuracy for the diagnosis of advanced fibrosis in NAFLD patients.

O021

NON-INVASIVE SCREENING OF LARGE ESOPHAGEAL VARICES USING ENDOSCOPIC CAPSULE AND/OR LIVER FIBROSIS TESTS

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Background and Aims: The non-invasive screening of large esophageal varices (LEV) might be useful in primary prevention of variceal bleeding in cirrhosis. The main objective was to compare strategies for the diagnosis of LEV by using predictive values and direct costs.

Methods: 330 cirrhotic patients were included in a prospective multicenter study. Diagnostic tools were upper gastro-intestinal endoscopy (UGIE, reference), esophageal capsule endoscopy (ECE), liver stiffness measurement (LSM) by Fibroscan and several blood markers and liver fibrosis tests.

Results: 287 patients had per protocol analysis defined by UGIE and ECE available. Child–Pugh class distribution was: A: 61%, B: 20%, C: 19%. Etiology was: alcohol: 45%, viral hepatitis: 27%, NASH: 5%, mixed: 11%, others: 6%. Among the 35 diagnostic predictors tested in the per protocol population, 10 had an AUROC >0.7 for LEV: Child–Pugh score (0.72), bilirubin (0.72), AST/ALT (0.74), LSM (0.74), hyaluronate (0.77), QuantiMeter (0.77), FibroMeter (0.77), CirrhoMeter (0.77), Elasto-FibroMeter (0.78), ECE (0.89). The most performant combination by binary logistic regression was either with ECE: ECE + AST/ALT (score 1, AUROC: 0.92) or without ECE: prothrombin index + AST/ALT (score 2, AUROC: 0.77), LSM having no independent role. Then, we defined 5 strategies based on non-invasive tools with high negative predictive value (NPV ≥95%) and/or positive predictive value (PPV ≥90%) for LEV in a first step; then, UGEI was used in the indeterminate zone. The 5 strategies were: S1: ECE for NPV and PPV, S2: LSM for NPV and ECE for PPV, S3: LSM for NPV and score 2 (ECE + blood markers) for PPV, S4: score 1 (blood markers) for NPV and PPV, S5: score 2 (ECE + blood markers) for NPV and PPV. The rank of strategies for decreased request to UGEI was: S5: 12.9%, S1: 28.9%, S2: 41.7%, S3:42.2%, S4: 47.5% ($p < 0.001$ between all and S5 vs others). But the rank for direct costs per patient was different: S4: 64€, S2: 393€, S3: 399€, S5: 632€, S1: 645€, reference UGEI cost being 114€.

Conclusions: This is the first study comparing capsule endoscopy and fibrosis tests in the screening of large esophageal varices. By using capsule and blood markers, one can avoid 87% of endoscopies in cirrhotic patients but this strategy has currently high direct costs (x6). By contrast, simple blood markers can avoid 48% of endoscopies and divide the direct costs by around 50%. These results should be validated in an independent population, compared to spleen stiffness and add indirect costs.

O022

THE UK-PBC RISK SCORE: DERIVATION AND VALIDATION OF A RISK SCORE TO PREDICT LIVER EVENTS IN THE UK-PBC RESEARCH COHORT

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Background and Aims: Outcomes in primary biliary cirrhosis (PBC) can be predicted by biochemical response to ursodeoxycholic acid (UDCA). However, stratification based on UDCA response does not take the stage of the liver disease into account. Furthermore, existing definitions dichotomise UDCA response and long-term risk, whereas both are a continuum. We analysed the UK-PBC Research Cohort to develop and validate a risk score that includes markers of disease stage, as well as the post-treatment liver biochemistries modelled as continuous variables

Methods: We constructed a PBC risk score for LT and liver-related death at 15 years in a derivation cohort (N = 2422) and evaluated it in a validation cohort (N = 1600). We used multivariable fractional polynomials (MFP) to model non-linear risk relations with continuous variables, and multiple imputation (20 imputations) to replace missing values. We fit a Cox proportional hazards model in each imputed dataset, and used Rubin's rules to combine the results. The resulting coefficients were used together with the baseline survivor function to derive an equation for absolute risk at 15 years. Net reclassification improvement (NRI) was calculated to compare the predictive performance of this risk score compared with other prognostic scores

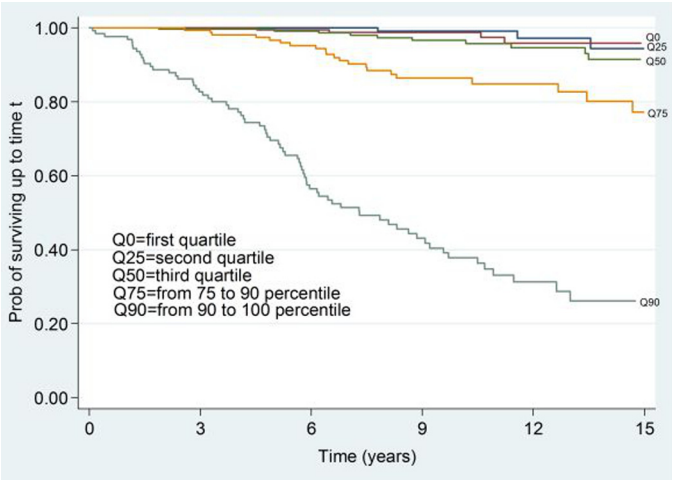

Figure 1.

Results: Median follow up time was 6.6 yrs (IQR, 3.3–11.1) and 537 patients reached the endpoint. The following variables were independently associated with a liver event: albumin and platelet

at baseline, ALP, bilirubin and transaminases after 12 months of UDCA. The score incorporated these five variables, appropriately transformed using MFP. When applied to the validation cohort, the score was highly predictive (c-statistic = 0.90). Figure 1 shows the KM survivor function according to risk groups of the score in the validation cohort. The NRI showed that the risk score had greater ability to identify individuals with and without events compared to other risk scores (NRI of PBC risk score vs. Paris1 = 90%; Paris2 = 64%; Barcelona = 38%; Toronto = 71%).
Conclusions: Prognosis of UK patients with PBC can be accurately assessed with the PBC risk score by using readily available objective clinical measures. This may be used to identify high-risk patients for closer monitoring and second-line therapies, as well as low-risk patients who require infrequent monitoring and might even be followed-up in primary care. However, validation of the score in an external independent cohort and identification of thresholds to inform clinical decision-making is required.

O023
REPEATED MEASUREMENT OF NON-INVASIVE FIBROSIS MARKERS TO ASSESS HEPATITIS C PROGRESSION AND CLINICAL OUTCOMES

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Background and Aims: Transient elastography (TE) and serologic non-invasive markers of fibrosis (NIMF) estimate disease stage in patients with chronic hepatitis C (CHC). Few data are available on the usefulness of consecutive measures to assess disease progression and particularly clinical outcomes.
Aims: To assess the prognostic value of consecutive measures of TE and NIMF (APRI, FIB4, Forns) in CHC patients without cirrhosis since the introduction of TE in 2004.
Methods: Clinical and biochemical data of consecutive non-cirrhotic CHC monoinfected patients were recorded. The time of first TE measure was considered *baseline* (BL-TE). Clinical events such as cirrhosis (LC), hepatocarcinoma (HCC), liver transplant (LT) or death were assessed during follow-up until 2014.
Results: 696 patients were included (51% male, median age 47 years, median BMI 19 kg/m^2, 4% diabetic). Genotype-1 and IL28B CT/TT were predominant (80% and 70%, respectively). Overall, median BL-TE was 6.5 kPa (P_{25}-P_{75} 5.4–8.3)] and ≥7 kPa in 41%. Median follow-up was 6.4 years (P_{25}–P_{75} 5–7) during which 60% received antiviral treatment [28% of them achieving sustained virological response (SVR)]. Sixty-eight patients (9.7%) developed LC, 7 HCC, 1 underwent LT and 2 died. After 3 years, TE and NIMF were obtained in 547 patients (78%). Patients achieving SVR or who developed LC before this time-point were censored. Delta TE (ΔTE) was calculated as (TE follow-up) − (BL-TE)/years. Mean ΔET was 0.06 kPa/year in the entire cohort, but it was significantly higher among patients who developed LC compared to those who did not (0.89 vs 0.02 kPa/year; $p < 0.001$). By Cox-Regression analysis, baseline FIB-4 (1.7 [1.3–2.2]), BL-TE≥7 kPa (6.5 [3.2–14]) and higher ΔTE (4 [2–7.7]) were associated with the development of liver events (LC/CHC). The best AUROC to predict events resulted from the combination of BL-TE and ΔTE (0.88), significantly higher than those obtained with BL-TE, Forns, FIB-4 or APRI (0.79, 0.74, 0.71 and 0.67, respectively) ($p < 0.001$). A new assessment was performed in 528 patients (76%) after 5 years, observing a mean ΔTE of 0.07 kPa/year. Again, ΔTE was significantly higher among patients who developed LC compared to those who did not (0.37 vs 0.05 kPa/year; $p < 0.001$).
Conclusions: The dynamic evaluation of liver fibrosis by TE is a useful tool to predict clinical outcomes in patients with CHC. Those patients with higher baseline TE and increase of TE value/year during follow-up, are at higher risk of liver events in the mid-term.

Liver immunology

O024
CYCLOPHILIN AND NS5A INHIBITORS, BUT NOT OTHER ANTI-HCV AGENTS, PRECLUDE HCV-MEDIATED FORMATION OF DOUBLE MEMBRANE VESICLE VIRAL FACTORIES

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Background and Aims: Recent advances in understanding molecular aspects of the HCV life cycle lead to successful development of several classes of anti-HCV agents including NS3 (NS3i), NS5A (NS5Ai) and NS5B inhibitors (NS5Bi) as well as host-targeting antivirals such as cyclophilin inhibitors (CypI). A number of these compounds are in IFN-free clinical trials and several are now FDA-approved. The mechanisms of action (MoA) of NS3i and NS5Bi are well understood, however the MoA of CypI and NS5Ai remain elusive. In this study, we conducted a set of experiments aimed to understand how these two classes of inhibitors exert their antiviral activity during HCV replication.
Methods: We examined whether CypI and NS5Ai interfere (i) with HCV RNA synthesis of isolated replication complexes (RCs) using a quantitative replicase assay, or (ii) with earlier steps of HCV RNA replication such as the creation of ER-derived double membrane vesicles (DMVs) essential for HCV RNA replication using a DMV counting assay based on EM analysis. Image J and ITEM software were used for DMVs quantification.
Results: In contrast to NS5Bi, we found that both CypI and NS5Ai do not block the HCV RNA synthesis by isolated RCs, suggesting that they exert their antiviral activity prior to the establishment of enzymatically active RCs. HCV replication is believed to occur at an ER-derived structure called the membranous web (MW), which is thought to be composed primarily of DMVs (200–300/cell). Viral replication is not a precondition for DMVs formation since we found that expression of the NS3-NS5B polyprotein or NS5A alone suffice to create DMVs. Using a panel of anti-HCV agents, we found that only CypI and NS5Ai, but not NS5Bi, mir-122 or PI4KIIIa inhibitors, prevent NS3-NS5B- or NS5A-mediated DMVs formation. We also found that NS3-NS5B and NS5A are unable to create DMVs in cyclophilin A (CypA)-knockdown cells.
Conclusions: These results not only suggest that CypA and NS5A act in concert to build these membranous viral factories, they also suggest that CypI and NS5Ai mediate their early anti-HCV effects by preventing the formation of specific organelles where viral replication normally occurs. This is the first comprehensive investigation to examine the effect of a large panel of anti-HCV agents on DMVs formation, and the results reveal that CypI and NS5Ai act at the same MW biogenesis step of HCV RNA replication, and indicate a new therapeutic target of the HCV life cycle.

O025
ROLE OF CX3CL1–CX3CR1 AXIS FOR LIVER DENDRITIC CELL MATURATION IN HOMEOSTASIS AND INFLAMMATION

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Background and Aims: Chemokines are critical regulators for liver inflammation and fibrosis. The CX3CL1/CX3CR1 chemokine pathway mediates chemotaxis, adhesion and survival of distinct

inflammatory leukocytes. CX3CR1 is expressed on multiple cell types including monocytes and dendritic cells (DCs). Recent evidence indicates that CX3CR1 deficiency exacerbates liver injury and fibrosis in CCl_4 treated mice, however, the mechanisms involved are still poorly understood. We aimed at elucidating the role of CX3CR1 for hepatic DC responses in homeostasis and acute liver injury.

Methods: $CX3CR1^{GFP/GFP}$ and $CX3CR1^{+/GFP}$ mice were injected intraperitoneally with CCl_4 (0–0.6 ml/kg) and liver mononucleated cells were analyzed by flow cytometry 36 hours later.

Results: In homeostasis, $CX3CR1^{GFP/GFP}$ mice showed a significant lower percentage of $CD11c^{high}$/MHCII$^+$/ $CX3CR1^{high}$ DCs in liver parenchyma ($p < 0.05$) than $CX3CR1^{+/GFP}$. Conversely, knockout mice displayed a higher percentage of liver plasmocytoid DCs $B220^+CD11c^+MHCII^+$ compared to $CX3CR1^{+/GFP}$. In line with the tolerogenic activity of liver DCs, CTLA4 expression by CD4+/CD25+ regulatory T-cells (Tregs) was lower in $CX3CR1^{GFP/GFP}$ mice, despite the overall number of hepatic $CD4^+$ T cells was comparable between the two strains. Recent evidence indicates that liver injury is associated with changes in hepatic DCs. CCl_4 treatment of wild-type and $CX3CR1^{GFP/GFP}$ mice lowered the pool of myeloid DCs and deeply affected the monocyte differentiation to DCs in the knockout strain. After CCl_4 treatment both $CX3CR1^{+/GFP}$ and $CX3CR1^{GFP/GFP}$ strains showed very strong liver recruitment of $CD11c^{high}$/$MHCII^+CD11b^+$ monocyte-derived DC (moDC). Interestingly, in $CX3CR1^{+/GFP}$ mice, these $CD11c^+MHCII^+CD11b^+CX3CR1^{high}$ moDCs had higher expression of the co-stimulatory proteins CD80 and CD40, indicating CX3CR1's involvement in moDCs maturation. However, CX3CR1 deficiency also affected the expression of immune regulatory PDL-1 on DCs ($p < 0.01$). Accordingly, CCl_4 treated $CX3CR1^{GFP/GFP}$ mice showed a higher expression of T-cell activation marker CD69 associated to a lowering in hepatic $CD4^+CD25^+Foxp3^+$ regulatory T cells.

Conclusions: These results suggest a novel role of CX3CL1/CX3CR1 axis in liver DC maturation/differentiation that may account for the increased severity of liver injury in CX3CR1-deficient mice.

O026

CD4+ INTRAHEPATIC LYMPHOCYTES DRIVE LIVER INFLAMMATION VIA IMPAIRED REGULATORY PATHWAYS IN A MURINE MODEL OF CROHN'S-LIKE ILEITIS

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Background and Aims: Crosstalk between the liver and intestines represents an important pathophysiological interface in inflammatory bowel disease (IBD), a disorder associated with significant hepatobiliary manifestations. We previously showed that liver inflammation precedes the onset of gut inflammation in a murine model of Crohn's disease, i.e., SAMP1/YitFc (SAMP) strain. The aim of this study was to determine the specific pathogenic mechanism(s) leading to chronic liver inflammation in ileitis-prone SAMP mice.

Methods: Liver inflammation was evaluated in reciprocal SAMP-AKR/J (AKR control strain) bone marrow chimeras (BMC), lymphocyte-depleted SAMP (SAMPXRAG-2 KO), and immunodeficient SCID mice receiving SAMP or AKR donor CD4+ cells. Isolated non-parenchymal liver cells (NPLC) were phenotypically-characterized by FACS and qPCR. Liver-resident CD4+ T effector cells (Teff) and T regulatory cells (Treg) were FACS-purified and functionally tested for in vitro proliferation and suppression activity.

Results: Construction of BMC revealed that, unlike SAMP ileitis that originates from a non-hematopoietic source, both non-hematopoietic and hematopoietic compartments are essential for liver inflammation; in fact, SAMPXRAG-2 KO mice displayed no hepatic inflammation vs SAMPXRAG-2 WT ($p < 0.001$), confirming a requirement for lymphocytes. Indeed, SAMP intrahepatic infiltrates displayed Th1-polarized, phenotypically-activated CD4+ T-cells vs AKR ($p < 0.05$). Moreover, SAMP vs AKR liver-derived CD4+ T-cells induced severe liver and ileal inflammation when adoptively transferred into SCID recipients ($p < 0.05$ and $p < 0.01$, respectively), whereas gut-associated lymphoid tissue-derived CD4+ T-cells produced ileitis ($p < 0.01$ vs AKR), but not liver inflammation. Expression of the gut homing molecules, MAdCAM-1 and CCL25, was unchanged in SAMP vs. AKR liver, suggesting that intrahepatic CD4+ T cells are not likely recruited from the gut. Interestingly, decreased frequencies of resident tolerogenic populations, i.e. liver sinusoidal endothelial cells ($p < 0.005$) and Treg ($p < 0.05$) were evident in SAMP vs AKR livers. Preliminary in vitro experiments displayed increased Teff proliferation and a marked decrease of Treg suppression activity in SAMP vs AKR liver.

Conclusions: These results challenge the current paradigm that IBD-associated liver inflammation is a consequential secondary event and suggest that intrahepatic $CD4^+$ T-cells acquire pathogenic potential in predisposed individuals through decreased liver-resident regulatory mechanisms.

O027

THE EXPRESSION OF TUMOR SUPPRESSOR PTPRD IS DOWN-REGULATED IN THE LIVER OF PATIENTS WITH HCV INFECTION AND IN TUMOR LESIONS OF PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Background and Aims: Chronic hepatitis C virus (HCV) infection is a leading cause of chronic liver disease including hepatocellular carcinoma (HCC). It is generally assumed that HCV contributes to HCC development directly by viral proteins and indirectly by signal transduction. Cellular signaling cascades are involved in cancerogenesis and the regulation of HCV. Signaling is tightly regulated by protein phosphatases and their aberrant expression is involved in various diseases and syndromes. However, the global impact of HCV infection on the expression of phosphatases and their impact on liver disease are unknown. Therefore, we aim to identify the impact of chronic HCV on protein phosphatase expression pattern

Methods: We studied the expression of 84 pathway or disease-focused phosphatases in liver biopsies of chronic HCV-infected patients using using real-time PCR, fluorescence *in situ* hybridization (FISH) and Western blotting.

Results: We identified 29 phosphatases that are significantly ($p < 0.01$, Mann–Whitney U-test) deregulated. Among the identified hits were several phosphatases associated with cancerogenesis including the tumor suppressor gene phospho-tyrosine phosphatase, receptor type D (PTPRD). PTPRD expression did not correlate with the METAVIR score of the studied biopsies suggesting that PTPRD expression is independent from tissue inflammation and fibrosis. PTPRD expression was impaired in HCV-infected liver tissue but not by HBV. Moreover, PTPRD was

downregulated in primary human hepatocytes (PHH) infected with HCVcc (JFH1), while IFN-alpha treatment had no impact on PTPRD. Taken together, the data suggest that HCV specifically and directly impairs PTPRD expression in hepatocytes independent from the innate immune response. Furthermore, we demonstrated that PTPRD expression is impaired in transformed liver cell lines and in tumor lesions of HCC patients, which we confirmed by *in silico* analysis of a microarray database (Hoshida et al. N. Engl. J. Med. 2008). These data demonstrate an impaired PTPRD expression associated with HCC.
Conclusions: Interestingly, PTPRD dephosphorylates STAT3, which is activated in the majority of HCCs with poor prognosis. We previously demonstrated that STAT3 has an important proviral effect on HCV infection (Lupberger et al., Hepatology 2013). It is thus conceivable that HCV downregulates PTPRD for its replication and immunoevasion with potential consequences for HCC development.

O028
CROSS-TALK BETWEEN IL13 AND HEDGEHOG PATHWAYS CONTRIBUTES TO SCHISTOSOMIASIS MANSONI FIBROSIS

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Background and Aims: Schistosomiasis mansoni is a major cause of liver fibrosis and portal hypertension. IL13 and Hedgehog (Hh) increase in schistosomiasis, regulating stellate cell activation and alternative activation of macrophages (M2). Our *aims* were to investigate if there is cross-talk between IL13 and Hh in murine and human schistosomiasis.
Methods: Male Balb/c wild-type mice (n = 9), IL13Rα1$^{-/-}$ mice lacking IL13 signaling (n = 9) and TKO [IL-10$^{-/-}$, IL12/23(p40)$^{-/-}$, IL13Rα2$^{-/-}$] mice with constitutive IL13 activity (n = 6) were infected with *Schistosoma mansoni*. Uninfected wild-type male Balb/c mice (n = 5) were controls. To investigate the effect of Hh on M2 polarization, primary murine kupffer cells (pmKC) were isolated from Smoothened$^{flox/flox}$ mice (Smo$^{flox/flox}$) by elutriation. Smo was knocked down using an adenovirus harboring Cre recombinase (or GFP control). 87 wedge liver biopsies from schistosomiasis patients (low fibrosis n = 15, severe fibrosis n = 72), 22 biopsies from noninfected transplant donors, 33 snap frozen wedge liver biopsies from patients with severe schistosomiasis and from 4 transplant donors were evaluated. IL13, Hh pathway (Ihh, Shh, Gli1, Gli2, Ptch1), fibrogenesis (Col6a1, aSMA), and M2 markers (Chi3l3, Arg1, Fizz1) were assessed by qRTPCR, Western blot and immunohistochemistry. Fibrosis was evaluated by Sirius red histochemistry and hydroxyproline content. This study was approved by the animal and human ethics committees of NIH, Duke, and UFMG.
Results: Infected TKO mice had more collagen, myofibroblasts, M2 macrophages and Hh pathway activity than infected wild-type mice, infected IL13Rα1$^{-/-}$ mice, and non-infected controls. Infected IL13Rα1$^{-/-}$ mice had lower expression of Ihh, Shh, Gli1 and Gli2 than uninfected controls, suggesting that IL13 promotes Hh signaling. Hh pathway activity correlated with collagen accumulation, M2 marker expression, and myofibroblast activation. Deletion of Smo in pmKC abrogated M2 polarization. Patients with severe schistosomiasis had increased IL13 and Hh pathway activity and Hh signaling correlated with collagen deposition and fibrosis staging by ultrasound.
Conclusions: IL13-mediated activation of Hedgehog promotes M2 polarization and liver fibrosis in schistosomiasis.

O029
HCV TRIGGERS WNT PARACRINE SIGNALLING THAT MODULATES METABOLIC LIVER ZONATION

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Background and Aims: Metabolic identity of a hepatocyte is determined by its position along the centro-portal axis of a liver lobule. This functional specialisation is called metabolic liver zonation and its initiation and maintenance is under the control of wnt/β-catenin signalling. Altered metabolic zonation is associated with many hepatic pathologies, including chronic hepatitis C. Interestingly, although only a minority of hepatocytes are infected, the liver undergoes major systemic metabolic changes, implying inter-cellular cross talk between the infected and the healthy cells.
Methods: Transgenic mice with hepatocyte-targeted expression of the totality of HCV proteins and needle biopsies from hepatitis C patients at the early stages of the disease were studied in respect to patterns of lipid metabolism and accumulation in the context of metabolic zonation of the liver lobule.
Results: Low levels of viral proteins are sufficient to drive striking alterations of hepatic metabolic zonation independently of inflammation or the immune response. In mice, a major lipogenic enzyme, fatty acid synthase, was redistributed from its normal periportal expression into the midzone of the lobule, coinciding with exquisitely specific midzone accumulation of lipids. Strikingly, alteration of zonation was not limited to lipid metabolism and appeared to be driven by systemic signalling by the Wnt/β-catenin pathway. Importantly, we show that similarly perturbed metabolic zonation appears to precede steatosis in early stages of human disease associated with HCV infection.
Conclusions: Our results rationalize systemic effects on liver metabolism triggered by a minority of infected cells, thus opening new perspectives for investigation of HCV-related pathology, notably in the context of increased risk of tumorigenesis.

O030
PRO-APOPTOTIC TNF RECEPTOR SIGNALING IS THE RESULT OF A NOVEL INNATE IMMUNE SENSING PATHWAY THAT DETERMINES CELL DEATH IN VIRUS-INFECTED HEPATOCYTES

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Background and Aims: Viral infection is detected by the innate immune system via pattern recognition receptors and by virus-specific CD8$^+$ T cells via (cross)-presentation of viral antigens. Many viruses have therefore developed mechanism to escape such immune recognition. We have previously identified a new CD8 T Cell effector function that accounts for 50% of antiviral T cell activity in viral hepatitis and overcomes immune escape by viruses that modulate MHC I presentation and conventional innate immune recognition. CD8 T effector cells (CTLs) secrete TNF upon recognition of viral antigens on cross-presenting liver sinusoidal endothelial cells which selectively kills infected hepatocytes. Here, we investigated the underlying mechanisms that render virus-infected hepatocytes susceptible to TNF-induced cell death.

Methods: We infected mice with a hepatotropic virus and challenged these mice by injecting TNF. Using knockout mice, as well as biochemical and multiparametric histological methods we analyzed the influence of different pattern recognition receptors. Additionally, we isolated mitochondria from virus-infected mice and performed metabolomic investigations to analyze the role of mitochondria in virus recognition as well as their role in apoptosis execution.
Results: We demonstrate that viral infection in hepatocytes is recognized even in the absence of classical pattern recognition receptor signaling. Rather, viral infection of hepatocytes modulates metabolic processes, leads to increased levels of the pro-apoptotic BCL-2 family members Bax and Bad that cause damage to mitochondria. This leads to a downregulation of the anti-apoptotic protein XIAP (X-chromosome linked inhibiting of apoptosis protein). Downregulation of XIAP facilitates TNF-induced activation of the initiator caspase 8 that together with increased mitochondrial sensitivity to activated caspase 8 is responsible for TNF-induced cell death in virus-infected hepatocytes.
Conclusions: We have identified a novel immune-sensing mechanisms in virus-infected hepatocytes that links effector molecules generated by virus-specific T cells to cell-autonomous induction of death selectively in virus-infected hepatocytes.

O031
TG1050, A NOVEL IMMUNOTHERAPEUTIC TO TREAT CHRONIC HEPATITIS B, CAN CONTROL HBsAg AND PROVOKE HBsAg SEROCONVERSION IN HBV-PERSISTENT MOUSE MODELS

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Background and Aims: Current therapies [nucleos(t)ide analogs (NUC) or pegylated-IFN-alpha] for chronic hepatitis B (CHB) virus infection rarely achieve virus clearance. Cohort studies have shown the critical role of cellular immune responses to control HBV infection. We developed a HBV-targeted immunotherapeutic called TG1050 and have shown that this Adenovirus based product encoding multiple HBV antigens in the frame of a large fusion protein is able to induce robust, multispecific, long-lasting and polyfunctional CD8+ T cells in different naive mouse models. We have now further characterized capacity of TG1050 to induce functional T cells and exert antiviral control in an HBV-persistent mouse model based on an adeno-associated virus recombinant for an over length HBV genome (AAV-HBV) causing the expression of infectious HBV particles in mouse livers.
Methods: Two mouse strains, HLA-A2/DR1 and C57BL/6J mice, were infected by the AAV-HBV. Following establishment of persistent HBV expression, TG1050 was injected once or multiple times and induced HBV-specific T cells were monitored along time (from 2 to 18 weeks post-vaccination) by IFN-gamma ELISPOT or intracellular cytokine staining. Viral parameters were followed in blood by qualitative or quantitative HBsAg, HBeAg, anti-HBs and anti-HBc ELISA assays and by RT-qPCR for HBV DNA quantification.
Results: In HBV persistent HLA-A2/HLA-DR1 mice, single and multiple TG1050 injections induce multispecific CD8 T cells producing multiple cytokines (IFNg, TNFa and/or IL2) detected in the liver at day 14 and 128 post-TG1050 injection. TG1050 exerts an early antiviral effect by controlling viral load and/or circulating HBsAg compared with control mice. In HBV-persistent C57BL/6J mice, multiple TG1050 injections induce multispecific CD8 T cells producing cytokines (IFNg and/or TNFa) detected 14 days after the first injection. A significant impact was observed on viral parameters with a sustained decrease in viral load, circulating HBsAg level (from 2 weeks and up to 3 months post-TG1050 injection). In this model, HBsAg/anti-HBsAg seroconversion was achieved in 30% of TG1050-injected mice.
Conclusions: Elimination of HBsAg and HBsAg seroconversion are the hallmarks of chronic HBV cure. We have shown here that TG1050, used in a stand-alone approach, can reach this key clinical goal, opening the road to clinical development. First-in-man study is currently being organized.

Alcohol and DILI

O032
STEROID THERAPY DIFFERENTIALLY ALTERS GUT MICROBIOTA COMPOSITION IN SEVERE ALCOHOLIC HEPATITIS

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Background and Aims: Chronic alcohol consumption leads to severe alcoholic hepatitis (SAH) with high 28 day mortality. It has been recently demonstrated that gut microbiota changes in individuals consuming alcohol. The standard of care in such patients is steroid therapy with response rates ~50%. Gut microbiota are known to play an important role in steroid metabolism. We hypothesized that at baseline and on therpy modulation of gut microbiota may influence the response to steroid therapy.
Methods: Consecutive patients with SAH [chronic alcohol use >5 years, DF >32, transjugular liver biopsy suggestive of SAH, absence of any known liver disease or h/o immunosuppressive drugs] were included and treated with prednisolone (40 mg/day) for 28 days. The response to streoids was assessed at day-7 by Lille score [<0.45 as responder (R), >0.45 as non-responder (NR)]. Stool samples were collected in sterile vials at baseline and day-7 of therapy and snap frozen until processed. 250 mg of frozen samples were processed for DNA isolation and with Primers for 16S rRNA gene V4 region. Samples were analyzed on MiSeq (Illumina).
Results: Stool samples were processed for 6-R and 6-NR for microbiome analysis. Total of 305 species belonging to 135 genera were identified. At baseline dominant species in NR were *Klebsiella* (32%) and *Enterococcus* (24%) in contrast to *Bacteroides* (26%) and *Lactobacillus* (21%) in R. After therapy, R showed a shift to *E. coli* (45%) and *Bifidobacterium* (21%); NR had *E. coli (40%)* and *Lactobacillus* (26%). Steroid therapy resulted in 6-fold increase ($p < 0.05$) in Proteobacteria in R and marginal reduction in NR. Members of Bacteroidetes reduced significantly ($p < 0.05$) after steroid treatment in R and NR. Similar observations were made in R for firmicutes, while the group demonstrated ~20% increase in NR. Actinobacteria (gram-positive) on the other hand were significantly ($p < 0.05$) increased (7-fold) in R and reduced (50%) in NR. Enterotype classification revealed responders to be type-1 and non-responders to type-2 at baseline. Post-therapy both groups shifted to type-3. It has been suggested that enterotypes are defined by diet. In our group while the diet of the patients was similar, the species composition of type-3 varied, suggesting an effect of steroid on species composition.
Conclusions: Gut microbial community is significantly different in the SAH patients. Gut microbiota also demonstrate differential compositional alterations in response to steroid.

O033

CCR2+ INFILTRATING MONOCYTES PROMOTE ACETAMINOPHEN-INDUCED ACUTE LIVER INJURY – THERAPEUTIC IMPLICATIONS OF INHIBITING CCR2 AND CCL2

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Background and Aims: Liver injury following acetaminophen (APAP) intoxication is one of the leading courses of acute liver failure (ALF). APAP causes necrosis of hepatocytes followed by an activation of resident immune cells like Kupffer cells (KC), release of various chemokines (e.g., CCL2) and immune cell infiltration (e.g., monocytes). We hypothesized that CCR2+ monocytes promote APAP-induced injury and investigated the therapeutic potential of pharmacologically blocking either CCR2 or CCL2.
Methods: C57BL/6J (WT) and *Ccr2*$^{-/-}$ mice were subjected to ALF by iv. injection of APAP (250 mg/kg body weight). Liver injury and immune cell phenotypes were analyzed in *Ccr2*$^{-/-}$ and WT mice, as well as in WT mice treated with mNOX-E36 s.c., a potent CCL2 inhibitor, or with the oral CCR2/CCR5 antagonist cenicriviroc (CVC). The human counterparts of both agents are currently tested in phase II trials for different indications (diabetic nephropathy or NASH, respectively).
Results: *Ccr2*$^{-/-}$ mice showed significantly reduced liver injury compared to WT mice 12 h after APAP injection as determined by histology and reduced ALT values ($p < 0.05$, Figure 1). Flow cytometry analyses revealed significantly reduced numbers of pro-inflammatory Ly6C+ monocyte-derived macrophages in livers of *Ccr2*$^{-/-}$ mice, whereas the numbers of neutrophils or other immune cell subsets remained similar to WT mice. While hepatic IL1β, TNF-α and CCL2 were similarly increased in both WT and *Ccr2*$^{-/-}$ mice, IL10 was higher in livers from *Ccr2*$^{-/-}$ mice ($p < 0.05$), alongside differential marker expression (CD1d, CD68) on hepatic macrophages. Both pharmacological inhibitors, mNOX-E36 or CVC, were capable of significantly reducing monocyte accumulation in APAP-injured livers, resulting in significant protection from liver damage (ALT levels, $p < 0.05$ for mNOX and $p < 0.01$ for CVC).
Conclusions: Targeting detrimental actions of pro-inflammatory monocytes by inhibiting either the chemokine receptor CCR2 or its ligand CCL2 (MCP-1) is a promising therapeutic option to restrict liver injury following an acetaminophen overdose.

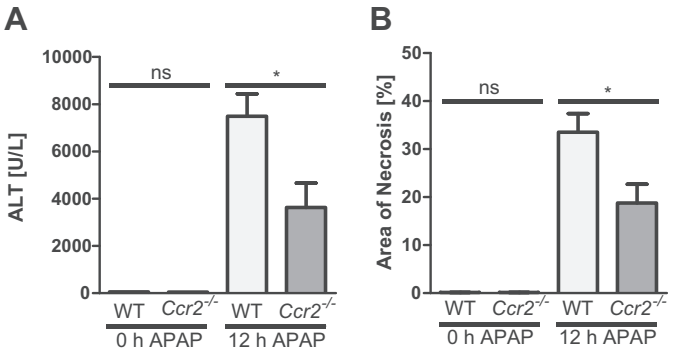

Figure 1. Acetaminophen-induced liver injury as determined by ALT (A) and histology (B) is significantly reduced in *Ccr2*$^{-/-}$ compared to WT mice.

O034

FREQUENCY AND FUNCTION OF ANTI-BACTERIAL MAIT CELLS ARE SIGNIFICANTLY IMPAIRED IN ADVANCED ALCOHOLIC LIVER DISEASE

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Background and Aims: Intestinal bacterial translocation and systemic gut-derived bacterial products are increasingly being shown to play a central role in the immunopathogenesis of alcoholic liver disease (ALD), yet the mechanisms of susceptibility to infection and the link with intestinal immunity remain elusive. Mucosal associated invariant T-cells (MAIT) are unconventional T-cells which only respond to bacteria-derived metabolites and are found in large numbers in the blood, intestinal mucosae and liver. They represent a key sentinel system for the homeostatic control of the gut flora and exert essential roles for control of bacterial infections. The aim of this study was to assess the role of MAIT cells in ALD.
Methods: Peripheral blood mononuclear cells from subjects with acute alcoholic hepatitis (AAH, Maddrey's discriminant function >32; n = 9), compensated alcohol-related cirrhosis (ARC, n = 9) and healthy controls (HC, n = 9) were examined by FACS. MAIT cells were identified as CD161+/Vα7.2+ CD8+ T-cells and MAIT-presenting cells (MPC) as MR1+ monocytes or B-cells. We quantified (1) frequency, activation status (CD69/HLA-DR) and immunoinhibitory signatures (PD1/TIM3/LAG3) of MAIT cells; (2) frequency and immunoinhibitory status (PD1/PDL1/TIM3/mGal9) of MPC; (3) cytokine/cytotoxicity profiles [IFNγ/TNFα/IL17; GranzymeB (GrB)/Perforin/CD107a] of MAIT cells after in-vitro stimulation with fixed *E. coli*. Plasma cytokines and endotoxin levels were assessed by ELISA.
Results: MAIT frequencies were reduced in ARC ($p = 0.005$) and dramatically lower in AAH ($p < 0.001$) compared to HC. MAIT cells from AAH and ARC were activated and hyperexpressed immunoinhibitory receptors compared to HC ($p < 0.03$). Frequencies of MR1+ MPC were comparable in all groups but expression of immunoinhibitory molecules was higher in AAH and ARC ($p < 0.03$). Membrane-bound MPC Gal9 was lower in AAH and ARC ($p < 0.01$) than HC whereas soluble Gal9 was higher ($p = 0.004$). Levels of *E. coli*-stimulated IFNγ from MAIT cells were comparable in all groups, but in ARC they produced less TNFα ($p = 0.024$) than HC. IL17 responses were observed only in HC. The cytotoxic potential of MAIT cells (GrB) was higher in AAH than HC.
Conclusions: This is the first report addressing the role of MAIT cells in liver disease. We show that MAIT cells have quantitative and functional impairments in ALD, with defective TNFα and IL17 responses, increased killing potential and hyperexpression of immunoinhibitory receptors. MAIT cells may represent a novel immunotherapeutic target for ALD.

O035

CCR9 AND CCL25 ARE EARLY PRO-INFLAMMATORY MEDIATORS IN ACUTE LIVER INJURY

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Background and Aims: Paracetamol is the most common of drug-induced acute liver failure (ALF). Paracetamol-induced ALF has a

significant associated mortality and few treatment options: liver transplantation remains the only treatment option for those not responding to medical treatment. $CCR9^+$ macrophages have recently been reported as playing a key role in a mouse model of acute liver injury. We investigated the CCR9/CCL25 receptor/ligand axis in acute liver injury.

Methods: Serum concentration of CCL25, the ligand for CCR9, was measured by ELISA in patients with ALF admitted to Kings College Hospital, London. Blood and liver-infiltrating immune cells were analysed by FACS. In murine models of ALF, mice were administered carbon tetrachloride (CCl_4) (1 ml/kg) or paracetamol (400 mg/kg) and sacrificed at specified time points. Liver injury was assessed by serum transaminase concentration.

Results: In patients with ALF, serum CCL25 concentration was markedly higher than healthy controls (mean 3.96 ng/ml vs. 0.878, $p < 0.001$). This was an early phenomenon with levels highest on the first day after admission and then decreasing ($p < 0.01$). $CCR9^+$ macrophages were identified in peripheral blood and explanted liver tissue of patients with ALF. In mice, liver-infiltrating macrophage numbers peaked within 24 hours of liver injury, which coincided with peak hepatic inflammation, measured by serum ALT levels. Expression of CCR9 on macrophages peaked between 6–12 hours and was greater in paracetamol than CCl_4 induced liver injury (mean percentage CCR9 expression 25.8% vs 11.0%).

Conclusions: The CCR9/CCL25 axis is upregulated early in both human and mouse acute liver failure. In mice, infiltration of liver tissue by $CCR9^+$ macrophages coinceides with the peak of necroinflammation as assessed by ALT. These data suggest a pro-inflammatory role for $CCR9^+$ macrophages in ALF.

O036

OVEREXPRESSION OF C-MYC IN HEPATOCYTES PROMOTES INITIATION AND PROGRESSION OF ALCOHOLIC LIVER DISEASE

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Background and Aims: Alcohol exposure may result in the overexpression of certain oncogenes in human cells thereby increasing the intracellular concentration of reactive oxygen species (ROS) and, thus, triggering initiation and progression of alcoholic liver disease (ALD). We previously showed that prolonged c-myc expression in hepatocytes leads to spontaneous fibrogenesis and end-stage high-latency tumor development. In the present study, we hypothesized that c-myc overexpression might exert a crucial role in the development of ALD.

Methods: Expression of c-myc was measured in biopsies of patients with ALD by quantitative real-time PCR (qPCR) and by immunohistochemistry (IHC). ALD in mice carrying transgenic over-expression of c-myc in hepatocytes (alb-myctg) and wildtype (WT) controls was induced by administration of control or ethanol (EtOH) Lieber DeCarli diet for 4 weeks. Primary hepatocytes were isolated from WT and alb-myctg mice, subjected to EtOH treatment and investigated for markers of cell cycle progression and oxidative stress by Western blotting, immunofluorescence and electron microscopy.

Results: Hepatic c-myc was strongly up-regulated in human patients with advanced ALD and in WT mice fed with EtOH-diet. Conversely, the over-expression of c-myc in hepatocytes led to early ballooning degeneration, increase of liver collagen deposition and ethanol-induced hepatic lipotoxicity, in conjunction with excessive CYP2E1-derived ROS after EtOH-diet. Unexpectedly, alb-myc^{tg}-livers displayed impaired cell proliferation resulting in remarkable hepatic hypertrophy and hepatocyte enlargement in response to EtOH challenge. Moreover, EtOH-fed alb-myc^{tg} mice exhibited profound dramatic changes in the mitochondrial morphology associated with mitochondrial dysfunction. Consistently, alb-myc^{tg}-derived primary hepatocytes showed blockade of proliferation and dramatic increase of cellular ROS, in response to EtOH challenge. Therefore, we explored the underlying molecular mechanisms, which demonstrated that in our experimental model of ALD, *c-myc* overexpression leads to strong activation of AKT, and, consequently phosphorylation of Mdm2 and degradation of p53 function.

Conclusions: Our findings show that the proto-oncogen *c-myc* accelerated the progression of ALD thereby increasing collagen deposition and intracellular concentrations of oxidants through a p53 pathway-depending mechanism. These results render *c-myc* as a plausible novel diagnostic and prognostic tool for early detection of ALD.

O037

MACROPHAGE AUTOPHAGY MEDIATED THE BENEFICIAL EFFECTS OF CANNABINOID RECEPTOR 2 IN ALCOHOLIC LIVER DISEASE

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Background and Aims: Kupffer cells play a major role in the pathogenesis of alcoholic liver disease. We have previously reported that during experimental ALD, the cannabinoid receptor 2 (CB2) limits Kupffer cells polarization towards a pro-inflammatory phenotype, thereby leading to decreased hepatic inflammation and steatosis. The aim of this study was to investigate the contribution of macrophagic CB2 receptor to these effects and the mechanisms involved.

Methods: Female mice were fed a Lieber-DeCarli liquid diet containing 5% ethanol for 10 days then gavaged with a single dose of ethanol (5 g/kg body weight) and sacrificed 9 hours later (NIAAA model). Experiments were performed in mice invalidated for CB2 receptor in the myeloid lineage ($CB2^{Mye-/-}$ mice) or in mice invalidated for the autophagy gene ATG5 in the myeloid lineage ($ATG5^{Mye-/-}$ mice) and treated with the CB2 agonist JWH-133. *In vitro* studies were performed on peritoneal macrophages isolated from WT or $ATG5^{Mye-/-}$ mice.

Results: As compared to WT littermates, $CB2^{Mye-/-}$ mice showed ennhanced alcohol-induced pro-inflammatory phenotype of Kupffer cells and hepatic steatosis. CB2 receptor activation by JWH-133 stimulated macrophage autophagy in the liver of alcohol-fed mice, whereas macrophage autophagy was inhibited in the liver of alcohol-fed $CB2^{Mye-/-}$ mice. These findings were confirmed in studies with cultured peritoneal macrophages, showing that autophagy is induced in macrophages exposed to JWH-133 and inhibited in CB2-deficient macrophages. Finally, JWH-133 reduced the induction of inflammatory genes by LPS in WT peritoneal macrophages, but not in ATG5-deficient cells. The CB2 agonist also protected from alcohol-induced liver inflammation and steatosis in WT, but not in $ATG5^{Mye-/-}$ mice demonstrating that macrophage autophagy mediated the anti-inflammatory and anti-steatogenic effects of CB2 receptor.

Conclusions: These results demonstrated that CB2 receptor activation in macrophages protects from alcohol-induced steatosis by inhibiting hepatic inflammation through an autophagy-dependent pathway in Kupffer cell.

O038

INVESTIGATING PARACETAMOL TOXICITY IN HepaRG-BASED 3D HUMAN HEPATIC ORGANOTYPIC MODELS WITH NON-INVASIVE OPTICAL COHERENCE PHASE MICROSCOPY (OCPM)

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Background and Aims: Conventional 2D primary human hepatocyte (PHHs) or HepG2/C3A cultures for drug testing rapidly lose polarity and differentiated function and poorly represents normal liver tissue. Human HepaRGs are the closest surrogate to PHHs – maintaining intact phase I–III drug metabolism. Our aim was to develop and test a more physiologically-relevant 3D hepatic organotypic co-culture platform for drug metabolism/ toxicology applications. Given established endpoint toxicity assays are often incompatible with assessing 3D tissues, we investigated the use of optical coherence phase microscopy (3D-OCPM) as a novel approach to assess cell viability in response to paracetamol hepatotoxicity.
Methods: HepaRG or HepG2/C3A (as comparators) cells, as 2D monolayers (controls), overlay ('2.5D') or hydrogel-encapsulated (3D), were prepared in either Matrigel (MG) or Hyaluronic acid hydrogels (HAH) in 96-well plates; and viability tested (LIVE/DEAD fluorescent staining). Paracetamol hepatotoxicity [0–40mM; 24 h] was assessed in optimal 3D cultures, with 3D-OCPM in-depth microstructural and functional imaging of 3D-encapsulated cells. This new approach was compared with established hepatotoxicity assays: Prestoblue (PB; Life Technologies), lactate release, and ATP content (Promega CellTiter-Glo-3D Real-Time Cell Viability) assays.

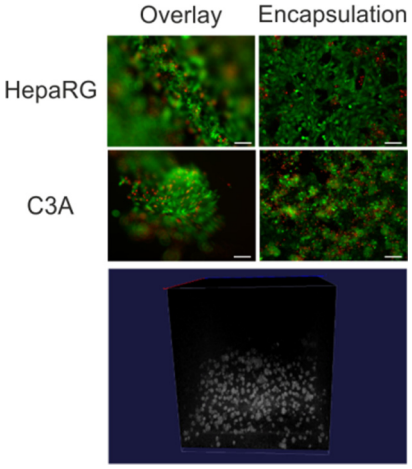

Figure 1. Upper panels: LIVE/DEAD (green/red) staining of 3D-encapsulated or 2.5D overlay HepaRG or C3A cells cultured in hyaluronan-based hydrogels with 'liver-like' compliance; Top right panel: viability = 95%. Lower panel: In-depth 3D microstructural imaging of hydrogel-encapsulated hepatocytes with optical coherence phase microscopy. Field of view 2×2×1 mm.

Results: Highest cell viability (Fig-1, LIVE/DEAD staining; PB assays) for optimal 3D culture was achieved using 3D HepaRGs encapsulated in HAH – and used for paracetamol hepatotoxicity. 3D-HepaRGs showed significantly less oxidative stress (lactate production; ATP-depletion) compared with all HepG2/C3A culture formats, and more resistant to paracetamol hepatotoxicity, in all 2D-3D culture configurations tested. 3D-OCPM (Fig. 1) was able to image at a microstructural level all encapsulated cells in 3D (in a 2x2x1 mm field of view), and the dose-dependent effect of paracetamol upon spheroid integrity. In addition, cell viability was retrieved from functional OCPM measurements and integrated over the volume investigated to yield a dose-dependency of paracetamol cytotoxicity on 3D liver cultures – that correlated with standard hepatotoxicity assays.
Conclusions: In this study we demonstrated 3D-HAH-encapsulated HepaRG organoids as a potential model for hepatotoxicity studies; and validated Optical coherence phase microscopy as a non-invasive enabling technology to assess hepatotoxicity in 3D liver models at the microstructural level.

O039

CIRCULATING EXTRACELLULAR VESICLES WITH SPECIFIC PROTEOME ARE NOVEL BIOMARKERS AND THERAPEUTIC TARGETS FOR ALCOHOLIC LIVER DISEASE

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Background and Aims: *Background:* Alcoholic liver disease (ALD) is one of the most common forms of chronic liver disease and includes a wide spectrum of disease from benign steatosis to steatohepatitis (ASH) to severe alcoholic hepatitis (AH). Noninvasive reliable tools that allow to determine the severity of liver damage and risk stratification as well as novel therapies for the severe forms are greatly needed. Our *aims* were to identify and characterize circulating extracellular vesicles, membrane bound vesicles released during cellular stress and key cell-to-cell communicators as potential novel non-invasive biomarkers and therapeutic targets for ALD.
Methods: C57/B6 mice were placed on the intragastric feeding model of continuous ethanol infusion for 4 weeks, or continuous ethanol infusion plus weekly binge for 8 week to reproduce a physiologically relevant model of mild ASH and AH, respectively. The extent of liver steatosis, inflammation, and fibrosis was assessed by histological and molecular analyses on liver specimens. Circulating extracellular vesicles (EVs) were isolated by ultracentrifugation from plasma and a complete characterization of EVs was performed by FACS, electron microscopy, dynamic light scattering and LC-MS/MS. Circulating EV biological function was investigated in bone marrow derived macrophage (BMDM) by FACS.
Results: We observed highly significant differences in the levels of circulating EVs in the plasma between control group and mild ASH ($p < 0.05$) or AH ($p < 0.01$). Extensive characterization of circulating EVs identified both microparticles (MPs) and exosomes (EXO) present in blood of mild ASH or AH mice. Proteomics analysis of circulating EVs from mild ASH detected various differentially expressed proteins compared to control mice. Finally, Incubation of BMDM with circulating EVs from mild ASH mice resulted in a significant up-regulation of various macrophage activation markers including, CD40 ($p < 0.05$), CD80 ($p < 0.05$), CD86 ($p < 0.01$), and MHCII, compared to control circulating EVs.
Conclusions: These findings uncover EVs as potential novel noninvasive biomarkers and therapeutic targets for ALD.

Fatty liver disease: Clinical

O040
HERITABILITY OF HEPATIC FIBROSIS AND HEPATIC STEATOSIS, AND THEIR SHARED GENE EFFECTS WITH METABOLIC TRAITS IN NAFLD: A PROSPECTIVE TWIN STUDY

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Background and Aims: Heritability of hepatic fibrosis is unknown and heritability of hepatic steatosis has not been systematically assessed in adults. *Aims:* To determine the heritability of hepatic fibrosis and steatosis, and their shared gene effects with metabolic traits in a prospective community-dwelling human twin cohort specifically designed to study genetics of NAFLD.
Methods: This is a *cross-sectional* analysis of a prospective cohort study of uniquely well-characterized community-dwelling twins in Southern California. All participants underwent a standardized research visit including history, physical examination, fasting laboratory tests, exclusion of other causes of liver disease and hepatic steatosis. The hepatic steatosis was quantified non-invasively by MRI determined proton-density-fat-fraction (MRI-PDFF) and hepatic fibrosis was quantified non-invasively by MR elastography (MRE) determined stiffness. NAFLD was diagnosed after exclusion of secondary causes of hepatic steatosis in those who had a MRI ≥5% consistent with NAFLD practice guidelines.
Results: 106 twins were screened and 96 were included in the present study. The average (±SD) age and BMI was 48.6 (± 20.6) yrs and 24.9 (± 5.8) kg/m^2, respectively. The mono-zygotic (MZ) twin-pairs showed a robust correlation in hepatic steatosis as quantifed by MRI-PDFF (r^2 of 0.77, p-value <0.0001) but not the di-zygotic (DZ) twin-pairs (r^2 of 0.19, p-value 0.5). Similarly, the MZ twin-pairs showed a robust correlation in hepatic fibrosis as quantified by MRE (r^2 of 0.43, p-value <0.015) but not the DZ twin-pairs (r^2 of 0.08, p-value 0.8). The heritability of hepatic steatosis (assessed by MRI-PDFF) was 0.67 (CI: 0.57–0.77, p-value $<1.1\times10^{-15}$) and the heritability of hepatic fibrosis (assessed by MRE) was 0.66 (CI: 0.47–0.85, p-value $<9.7\times10^{-14}$), respectively. Genetic co-variance assessment revealed a significant association between hepatic steatosis and BMI (p-value <0.01) and hyperinsulinemia (p-value $<10^{-8}$), and between hepatic fibrosis and HbA1c (p-value <0.001). There was no significant shared gene effect between hepatic steatosis and hepatic fibrosis.
Conclusions: Utilizing a uniquley, well-characterized cohort of community-dwelling twins, this study demonstrates that both hepatic steatosis and hepatic fibrosis are heritable traits. Although both are heritable, they appear to have distinct basis for their genetic susceptibility.

O041
INCREASING ASSOCIATION OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) WITH HEPATOCELLULAR CARCINOMA IN THE UNITED STATES: DATA FROM SURVEILLANCE, EPIDEMIOLOGY AND END RESULTS (SEER)-MEDICARE REGISTRIES (2004–2009)

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Background and Aims: HCC is increasingly reported in patients with NAFLD. Our aim was to assess the incidence and mortality of patients with HCC and NAFLD.
Methods: We examined SEER registries (2004–2009) with Medicare linkage claims files for patients with HCC. HCC cases were identified by the ICD-O-3 codes using topography and morphology codes 8170–8175. The linked Medicare data was used to identify NAFLD, hepatitis C (HCV), hepatitis B (HBV), alcoholic liver disease (ALD), and other liver disease by ICD-9-CM codes. NAFLD was defined by ICD-9 codes and by clinical diagnosis (cryptogenic cirrhosis, obese diabetics). Logistic regression model was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for risk of HCC. In addition, adjusted hazard ratios (HRs) for within one-year mortality were estimated by Cox proportional hazard regression model in the HCC.
Results: After exclusion criteria, 7,255 HCC cases and 21,765 controls without HCC remained. Of the HCC cases, 43% (n = 3,128) were related to HCV, 16% (1,133) to NAFLD, 13% (975) to ALD and 7% (542) to HBV. Of HCC cases, 16% (1,194) did not have a liver disease diagnostic code. Across the six-year period, there was an increasing trend in the number of HCC cases, from 928 (287 per 1000 cases) in 2004 to 1,464 (346 per 1000) in 2009, a 4% annual increase. The prevalence HCC in HCV increased by 8% (from 110 per 1000 in 2004 to 156 per 1000 in 2009) with a 5% increase in NAFLD (from 44 per 1000 in 2004 to 55 per 1000 in 2009), all p < 0.001. Compared to HBV/HCV patients with HCC, NAFLD with HCC patients were older (74±8 vs. 68±11 yrs), more likely to be White, had shorter survival time, higher tumor stage and more heart disease (all p < 0.0001). Also, 1-year mortality of HCV/HBV with HCC was about 50%, while it was 70% NAFLD with HCC (p < 0.0001). Of those who received a transplant after HCC diagnosis (n = 508); only 4% was related to NAFLD/HCC. In multivariate analysis, NAFLD increased the risk of HCC (OR: 95% CI = 6.32: 5.55–7.19) as compared to the controls without liver disease. Additionally, older age, lower income, un-staged HCC were associated with higher risk of 1-year mortality while receiving liver transplant and having localized tumor stage were protective against the risk of 1-year mortality (all p < 0.05).
Conclusions: NAFLD is becoming a major cause of HCC in the U.S. NAFLD HCC is associated with shorter survival time, more advanced tumor stage and lower possibility of liver transplant.

O042
WEIGHT LOSS INTENSITY IS STRONGLY ASSOCIATED TO IMPROVEMENT OF HISTOLOGICAL PARAMETERS IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS AFTER 52 WEEKS OF LIFESTYLE MODIFICATION

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Background and Aims: The effect of weight loss (WL) intensity on different histological features of nonalcoholic steatohepatitis

(NASH) remains unclear. Thus, we evaluated the effect of different cutoffs of WL percentage on individual histological parameters in patients with NASH treated with lifestyle changes during 52 weeks.

Methods: This was a prospective study performed in 293 subjects with histologically proven NASH treated in clinical practice with low-fat hypocaloric diet and increased physical activity for 52 weeks. At baseline, patients were classified as having definite NASH and excluded those with borderline NASH and cirrhosis. NAFLD activity score (NAS) and their components, and fibrosis score were computed. A paired liver biopsy at 52 weeks was performed to determine a minimum two-point improvement in NAS or NASH resolution without fibrosis impairment. Furthermore, changes on individual components of NAS and fibrosis were recorded. A blinded central review of biopsies was performed by two experienced pathologist. At the end of 52 weeks, patients were stratified in subgroups according to different weight loss percentage cutoffs (<1%, 1–3%, 3–5%, 5–7%, 7–10% and >10%).

Results: A total of 261 patients underwent paired biopsies. A significant WL > 7% was achieved in 54 of 293 patients. Although NAS improvement and resolution were observed in all subgroups with WL, these were more remarkable in those with WL > 7%, however, no significant differences were detected between those with WL 7–10% (72% and 83%, respectively) and >10% (88% and 83%, respectively). While subjects with WL > 7% experienced higher rates of ballooning and lobular inflammation improvement, these were similar for those achieving 7–10% (88% and 86%, respectively) or >10% (96% and 90%, respectively). Mild reductions in the weight were associated with notable steatosis improvement, thus, patients with 3–5% of WL showed 65% of steatosis improvement. Of the 29 individuals with WL > 10%, 13 (45%) and 12 (42%) reduced the portal inflammation and fibrosis scores. No significant difference in these parameters were seen across the subgroups with WL < 10%.

Conclusions: The intensity of weight loss induced by lifestyle changes is strongly associated to the grade of improvement of histological features of NASH. A weight loss >7% has a positive impact in most of the histological parameters; however, >10% is necessary to induce fibrosis and portal inflammation improvements.

O043

THE FNDC5 RS3480 IS PROTECTIVE ON THE STEATOSIS AND FIBROSIS IN PATIENTS WITH NAFLD

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Background and Aims: Irisin, the cleaved extra-cellular part of the protein Fibronectin type III domain-containing protein 5 (*FNDC5*), is released by muscles during exercise through PGC1alpha activation and could have a favourable metabolic action. The aim of this study was to investigate the potential association of *FNDC5* Single Nucleotide Polymorphisms (SNPs) with histological severity of Non Alcoholic Fatty Liver Disease (NAFLD) in European Caucasian patients.

Methods: Two large cohorts of patients ["discovery" (n = 304) and "validation" (n = 309)] with histologically characterized NAFLD (SAF score) were studied. Four *FNDC5* SNPs (rs3480, rs1683198, rs1746661, rs1298190) and the patatin-like phospholipase domain containing 3 (*PNPLA3*) rs738409 variant were genotyped by allelic discrimination using TaqMan reagents (Applied Biosystems Inc., USA). Multivariate logistic regression analysis (additive model) was conducted incorporating biologically relevant covariates that were associated with risk of NAFLD progression [age, gender, Body mass index, presence of type 2 diabetes mellitus (T2DM) and *PNPLA3* rs738409 genotype] to test the genetic association.

Results: The discovery and validation cohorts differed in some characteristics including gender mix (Female gender 82% vs 40%, $p < 0.0001$), BMI (42.9 vs 34.3, $p < 0.0001$), age (40.9 vs 50.4, $p < 0.0001$), prevalence of T2DM (20.1 vs 41.1%, $p < 0.0001$) and the histological grade of activity score (≥A1 36.2 vs 71.2%, $p < 0.0001$). However, degrees of steatosis (≥S2 59.5 vs 70.6%, p = NS) and stage of fibrosis (≥F2 28.6 vs 40.1%, p = NS) were not significantly different between cohorts. Of the four *FNDC5* SNPs tested, only rs3480 was significantly associated with steatosis and fibrosis in the discovery cohort. In multivariate analysis, carriage of *FNDC5* rs3480 minor allele was protective against advanced steatosis in both the discovery and validation cohorts as well as the combined cohorts ($\beta = 0.69 \pm 0.13$, 95% CI 0.54–0.89; P = 0.0036). Although no association with histological activity was seen, carriage of *FNDC5* rs3480 was also protective regarding the degree of fibrosis in the combined cohort ($\beta = 0.73 \pm 0.13$, 95% CI 0.57–0.95; P = 0.0172).

Conclusions: Carriage of the *FNDC5* rs3480 minor G allele is associated with less severe steatosis and milder fibrosis in a population of European Caucasian patients. Further work is ongoing to determine the mechanisms through which this variant mediates its effect.

O044

DISTINCT FECAL AND PLASMA BILE ACID METABOLOME OF MICROBIAL ORIGIN CHARACTERIZES HUMAN NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is the fastest growing global liver disease with two well described phenotypes: nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). Bile acid (BA) metabolism is emerging to be an important pathophysiologic determinant and a potential therapeutic target in the more aggressive phenotype NASH. We hypothesized that NASH is associated with dysregulated BA metabolism.

Aims: (1) To characterize plasma and fecal BA metabolome in NAFL and NASH compared to healthy controls, and (2) to test and validate BA metabolite as a potential biomarker to distinguish NASH.

Methods: Healthy controls and subjects with histologically proven NAFL and NASH were recruited after informed consent. Clinical, demographic and pertinent medical information was collected. Plasma and stool samples were also collected per protocol. High throughput mass spectrometry was used for metabolomics. Welch t-test, and multiple group comparisons by ANOVA were applied. Random forest classification and mean decrease accuracy was measured for biomarker detection in initial training set followed by testing in validation cohort.

Results: Training (n = 51, control 7, NAFL 13, NASH 31) and validation (n = 32, control 17, NAFL 9, NASH 6) cohorts were age and gender matched for the three study groups.

Plasma BA metabolome changes: Despite no significant changes in the training cohort, compared to the controls primary BA taurocholate was significantly decreased in NASH by 3.28 fold ($p < 0.05$) in the validation cohort. NAFL had significantly higher glycocholate ($p = 0.009$) and taurocholate ($p = 0.01$) compared to the controls. Plasma glycoursodeoxycholate was significantly increased in both NAFL ($p = 0.026$) and NASH ($p = 0.03$). Secondary conjugated

bile acid tauroursodeoxycholate was 4 and 17 folds increased in NASH (p = 0.08) and NAFL (p = 0.05) respectively vs. control.
Fecal BA metabolome: Several secondary BA were significantly altered in the training and validation cohorts. Glycodeoxycholate (GDCA) emeerged as the sole concordant marker that was significantly increased in NASH compared to NAFL and control groups. Interestingly, GDCA is a secondary BA produced by the action of enzymes existing in the microbial flora of the colon and promotes apoptosis.
Conclusions: NASH is characterized by distinct fecal and plasma BA metabolome with microbial metabolite GDCA as a potential fecal biomarker and future therapeutic target.

O045
BIOACTIVE LIPIDS IN THE HUMAN LIVER IN 'COMMON NAFLD' AND 'PNPLA3 NAFLD'

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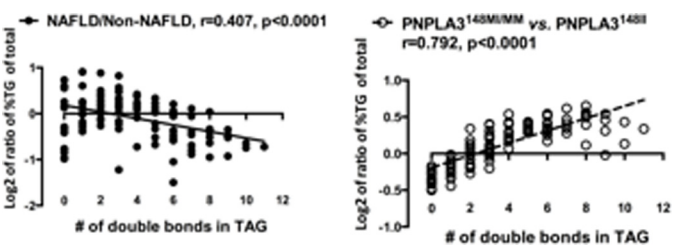
Background and Aims: 'Common NAFLD' is associated with hepatic insulin resistance and features of the metabolic syndrome while NAFLD due to the PNPLA3 I148M gene variant ('PNPLA3 NAFLD') is associated with increased liver fat but not insulin resistance. We hypothesized that this could be due to a difference in insulin resistance-inducing bioactive lipids, especially ceramides and diacylglycerols (DAGs) between the two types of NAFLD.
Methods: Liver biopsies from 126 subjects undergoing bariatric surgery were taken for profiling of molecular lipids using UPLC-MS. Liver histology, metabolic features and PNPLA3 genotype at rs738409 were also assessed. Subjects were divided into groups based on the presence of NAFLD ('Common NAFLD', n = 69, vs. 'Non-NAFLD', n = 57) and the PNPLA3 genotype (PNPLA3148MI/MM, n = 61 vs. PNPLA3148II, n = 65).
Results: Liver triacylglycerol (TAG) content was significantly increased in both types of NAFLD. The 'Common NAFLD' group was insulin-resistant compared with the 'Non-NAFLD' group (HOMA-IR 3.8 [2.3–5.4] vs. 2.6 [1.3–3.9], p < 0.0005), while HOMA-IR was similar in PNPLA3148MI/MM and PNPLA3148II groups (3.2 [1.7–5.1] vs. 3.2 [1.9–4.6], NS). Cluster analyses showed that the liver in the 'Common NAFLD' group was enriched in saturated and monounsaturated TAGs, ceramides and DAGs. Hepatic TAGs in the PNPLA3148MI/MM group were characterized by increases in polyunsaturated TAGs and no changes in ceramides and DAGs. Consequently, the number of double bonds in TAGs was negatively correlated with the fold-change of relative TAG ratios between the 'Common NAFLD' and the 'Non-NAFLD' groups (r = −0.407, p < 0.0001) but not between PNPLA3148MI/MM and PNPLA3148II groups (r = 0.792, p < 0.0001) (Figure).



Conclusions: The human liver lipidome differs markedly between 'Common NAFLD' and 'PNPLA3 NAFLD'. Lack of insulin resistance in 'PNPLA3 NAFLD' could be attributed to lack of an increase in saturated TAGs and accompanying increases in ceramides and DAGs.

O046
SHORT-TERM VITAMIN D SUPPLEMENTATION IMPROVES HEPATIC STEATOSIS AS QUANTIFIED BY CONTROLLED ATTENUATION PARAMETER (CAP)

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries. A recent meta-analysis has confirmed decreased serum 25-hydroxyvitamin D levels in patients with NAFLD. This intervention study investigates whether vitamin D therapy ameliorates hepatic steatosis in patients referred to the outpatient liver clinic of a tertiary centre.
Methods: We prospectively recruited patients with NAFLD and vitamin D deficiency, as defined by serum concentrations <20 ng/ml. Hepatic steatosis was assessed using the controlled attenuation parameter (CAP), which quantifies the degree of ultrasound attenuation by liver fat during vibration-controlled transient elastography (FibroScan). Patients were included if they had significant liver fat accumulation, which we defined by a CAP score ≥280 dB/m. Serum 25-hydroxyvitamin D were measured by chemiluminescent immunoassays. Body composition was determined with bioelectrical impedance analysis (TANITA BC-418 MA). Currently, 32 patients entered the intervention arm of the study and received 20,000 IU vitamin D daily for 7 days, thereon weekly for 6 months, while vitamin D, liver function tests, BMI and CAP levels were monitored.
Results: Overall, the cohort comprised 48.7% women (mean age 54±12 years; mean BMI 29.5±3.1 kg/m^2). Moderate vitamin D deficiency was present in 59.5% and severe vitamin D deficiency (<10 ng/ml) in 40.5% of patients. Fatty liver quantification showed a mean CAP of 328.5±30.9 dB/m. The CAP score was significantly lower in patients with severe vitamin D deficiency (342.8±31.8 *vs.* 317.3±27.0 dB/m, P = 0.013). A rapid increase in vitamin D levels was noted after 4 weeks of vitamin D supplementation (33.2 *vs.* 10.9 ng/ml, P < 0.0001), with 73% of patients displaying normal levels (>30 ng/ml). CAP scores significantly decreased by 5% at the 4-week interval (312.8 *vs.* 328.5 dB/m, P = 0.047). During this time-frame LFTs, BMI and body fat levels remained unchanged. Patients will be monitored again at 3 and 6 months.
Conclusions: Vitamin D levels correlate with the degree of hepatic steatosis, which significantly improves after only 4 weeks of vitamin D replacement therapy. We conclude that hepatic steatosis as assessed by CAP is a dynamic process, which appears to be modulated by short-term therapeutic interventions such as vitamin D substitution. The molecular mechanisms underlying hepatocellular lipid remodelling by vitamin D remain to be identified.

O047
REMOGLIFLOZIN ETABONATE REDUCES INSULIN RESISTANCE AND LIVER FUNCTION ENZYMES: ROLE FOR TREATMENT OF NASH

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Background and Aims: The etiology of non-alcoholic steatohepatitis (NASH) is due, in part, to insulin resistance and oxidative stress resulting from steatosis. To date, studies with anti-diabetics and anti-oxidants have resulted in small effects on reversal of these factors in NASH patients. Remogliflozin etabonate (Remo) is an SGLT2 inhibitor shown to reduce HbA1c in type 2 diabetics. We show here that Remo significantly reduces insulin resistance and,

Table 1 (abstract O047).

	Placebo	Remoglifozin etabonate (BID)					Pio (QD)
		50 mg	100 mg	250 mg	500 mg	1000 mg	
n [a]	35	37	38	36	40	37	38
ΔHOMA IS [b] (LOCF)	3.4	6.3	27.1	23.2	29.8	32.6	13.2
ΔHOMA BCF [b] (LOCF)	−2.5	23.1	17.9	26.2	33.4	42.7	38.2
n [a]	9	8	12	8	10	14	7
ΔAUC OGTT [c] (LOCF)	−0.4	−3.2	−2.6	−3.6	−3.5	−4.0	−3.0
ΔΔ Mean (95% CI) [d]		−2.8 (−4.7, −0.9)	−2.1 (−3.8, −0.4)	−3.1 (−5.0, −1.3)	−3.1 (−4.9, −1.3)	−3.6 (−5.2, −1.9)	−2.6 (−4.6, −0.7)

[a] Number of subjects with a value at baseline and at Week 12 (after LOCF).
[b] Model adjusted % change from baseline using log transformed data.
[c] Model adjusted change from baseline.
[d] Difference from placebo.

unlike other SGLT2 inhibitors, has intrinsic anti-oxidant activity, which may reverse the steatohepatitis and oxidative stress required to progress and maintain NASH.

Methods: A 12-week, double-blind, randomized, active and placebo-controlled trial was conducted with 336 treatment naïve subjects with type 2 diabetes and an HbA1c of >7.0% to <9.5% where the primary endpoint was change from baseline in HbA1c. Subjects were equally randomized to each of the Remo treatments (50, 100, 250, 500, or 1000 mg bid), matching placebo or 30 mg qd pioglitazone. Chemistry laboratories including LFTs, HOMA

Results: At Week 12, Remo improved both insulin sensitivity and beta cell function by 6–39% and 23–43%, respectively (Table 1). A significant decrease from baseline in AUC (0–2 hour) weighted mean plasma glucose following the OGTT was observed for all Remo treatment groups (Table 1). Post-hoc analysis of changes in alanine aminotransferase (ALT) indicated that subjects with elevated ALT at the Baseline (Week 0) showed 42–32% reductions at Week 12. Additionally, unlike other SGLT2 inhibitors, Remo has significant anti-oxidant activity as measured by the oxygen radical antioxidant capacity (ORAC) assay and reduces serum markers of oxidative stress in animal models of steatohepatitis.

Conclusions: Remo is a safe and potent anti-diabetic compound in late stage clinical development. Given its ability to reverse insulin resistance, lower ALT, and provide anti-oxidant activity, remogliflozin etabonate may be a useful treatment for NAFLD and NASH.

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General Session 2 & Award Ceremony 1

G07

THE PHASE 3 C-EDGE TREATMENT-NAÏVE (TN) STUDY OF A 12-WEEK ORAL REGIMEN OF GRAZOPREVIR (GZR, MK-5172)/ ELBASVIR (EBR, MK-8742) IN PATIENTS WITH CHRONIC HCV GENOTYPE (GT) 1, 4, OR 6 INFECTION

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Background and Aims: Safe, efficacious, and convenient antiviral regimens without interferon or ribavirin are being developed for chronic HCV infection. The C-EDGE TN study (P060) investigated the safety and efficacy of a once daily regimen of GZR (NS3/4A protease inhibitor) and EBR (NS5A inhibitor) for 12 weeks (wks) in TN patients (pts) with GT 1, 4, or 6 infection.
Methods: C-EDGE TN is an international, randomized, blinded, placebo-controlled, parallel-group trial of an oral fixed-dosed combination of GZR 100 mg/EBR 50 mg once daily in TN, HCV GT 1-, 4-, or 6-infected pts. Cirrhotic patients were eligible. Exclusion criteria included decompensated liver disease, HCC, HIV or HBV co-infection, platelets $<50\times10^3/\mu L$, or albumin <3.0 g/dL. Pts were assigned in a 3:1 ratio to receive either immediate or deferred therapy after stratification by GT and fibrosis stage assessed by biopsy or noninvasive means. HCV RNA levels were measured by the COBAS TaqMan v2.0 assay. The primary efficacy endpoint was prespecified as the proportion of treated pts in the immediate GZR/EBR arm with unquantifiable RNA levels (<15 IU/mL) 12 wks after the end of study treatment (SVR12).
Results: Overall, 421 (90%) of 469 screened pts were enrolled, randomized, and treated with ≥1 dose of study drug: 194 (46%) women; 157 (37%) non-white; 385 (91%) GT1 and 36 (9%) GT4/6 infections. Among the 92 (22%) pts with cirrhosis, the diagnosis was biopsy-proven in 26 (28%); the median platelet count [IQR] and albumin level [IQR] in cirrhotics were 123.5 [91.5–157.5] $\times10^3/\mu L$ and 4.1 [3.8–4.4] g/dL, respectively. All pts have reached follow-up (F/U) Wk4. Based on preliminary results from 315 GZR/EBR recipients in the immediate treatment arm, 306 (97%) achieved SVR4. Virologic failure occurred in 5/315 (1.6%) pts: 1 GT1a breakthrough at treatment Wk8 and 4 relapses (2 GT1a, 2 GT6) by F/U Wk4. There were 4 (1.3%) additional pts not achieving SVR4: 2 drug-unrelated deaths (incarcerated hiatal hernia and cardiac arrhythmia), 1 drug-related AE (palpitations/anxiety with drug discontinuation on treatment Day 4), and 1 loss to F/U. Serious AEs occurred in 10 (3.2%) and 3 (2.9%) pts in the active (immediate treatment) and placebo (deferred treatment) arms, respectively.
Conclusions: Early results suggest GZR/EBR for 12 wks is efficacious and well tolerated in TN pts with GT 1, 4, or 6 infection, including pts with compensated cirrhosis. SVR12 rates and full safety data will be presented.

G08

INCIDENCE OF HEPATOCELLULAR CARCINOMA (HCC) AND COMPLICATIONS IN ALCOHOLIC COMPENSATED CIRRHOSIS. PRELIMINARY RESULTS OF A MULTICENTER PROSPECTIVE FRENCH AND BELGIAN COHORT (INCA CIRRAL)

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Background and Aims: To assess the incidence of complications, mainly HCC, in alcoholic compensated cirrhosis.
Methods: Patients with histologically proven alcohol-related cirrhosis, Child–Pugh A, no HBV or HCV infection and no previous HCC were included then prospectively screened for HCC. At inclusion then yearly, biobanks and breath ethanol concentrations were colected.
Results: From october 2010 to september 2014, 538 eligible patients were consecutively enrolled in 20 active centres of whom 46 were excluded from the analysis (mainly because of absence of liver biopsy n = 7, Child–Pugh ≥B7 n = 21, or HCC n = 6). Of the 492 patients [median age 59 yrs, males 67%, seniority of the cirrhosis: 28.5 months, previous decompensation 65%], 304 (65%) were completely abstinent (median duration for alcohol withdrawn: 28.8 months) and 73 (15%) had still a mild consumption <7 glasses/week (absence of ethanol in breath for 309/378 patients at enrolment, 82%). Most patients were smokers (past-history of tabacco 35%, active smokers 39%) and regular consumption of coffee [78%; median: 2 cups/day; ≥3 cups/day in 123/317 (39%)]. Metabolic syndrome defined according to International Diabetes Federation (2005) was present in 84 patients (21%). At enrolment, 21 patients (4%) had a liver nodule (hemangioma n = 3, biliary kyste n = 7, regenerative nodule n = 1, miscellaneous n = 10) that was not

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anymore described during follow-up in 13 of them. Based on a median follow-up of 12.4 months: (i) liver nodule(s) occurred in 50 patients (1-yr cumulative incidence: 11%) diagnosed as HCC in 14 cases (1-yr cumulative incidence: 1.3%) without any statistically difference according to alcohol consumption or the presence of metabolic syndrome; description of the baseline characteristics of HCC ongoing; (ii) 160 events besides HCC were censored in 90 patients (1-yr cumulative incidence: 18%) related to a complication of cirrhose in half of cases; (iii) 28 deaths occurred directly attributable to the liver disease in 54% cases.

Conclusions: Early results of this prospective cohort are: (a) only 28% of detected liver nodules were confirmed as primary liver cancer; (b) after a follow-up of 1 yr, liver-related mortality already had concerned more than one half of deaths. Additional enrollments are ongoing. This cohort constitutes the backbone permitting precise study of HCC and other complications of cirrhosis, particularly through subsequent follow-up and nested studies exploiting high quality clinical data and biobank.

G09

TARGETING A HOST-CELL ENTRY FACTOR BARRICADES ANTIVIRAL RESISTANT HCV VARIANTS FROM ON-THERAPY BREAKTHROUGH IN HUMAN-LIVER MICE

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Background and Aims: Both viral and host proteins are involved during the hepatitis C virus (HCV) life cycle. Direct Acting Antivirals (DAAs) inhibit HCV infection by targeting viral proteins and are successfully used to treat HCV infections. However, therapy failure caused by the emergence of DAA-resistance associated variants (RAVs) remains a reasonable concern. Presumably, future anti-HCV therapies will consist of drug combinations that target distinct steps of the viral life cycle. The conserved host factors used by the virus for its propagation seem interesting alternative targets for antiviral intervention, complimentary to the action of DAAs.

Methods: We have previously shown that monoclonal antibodies (mAbs) against the HCV co-receptor scavenger receptor class B type I (SR-BI) inhibit HCV infection of different genotypes in human-liver chimeric mice, even when therapy was initiated several days after exposure. In addition we have recently shown that viral variants with *in vitro* reduced dependency on SR-BI can still be successfully blocked *in vivo* by our mAb. These observations suggested that anti-SR-BI mAb1671 efficiently prevents intrahepatic viral spread. Therefore we hypothesized that mAb1671 could prevent on-therapy breakthrough of DAA-RAVs, by restricting DAA-RAVs to the hepatocytes in which they are selected, thereby improving virologic response. Thirteen gt1b-infected mice were randomized over two study groups. Seven mice received BILN-2061 (Ciluprevir) monotherapy, while the six remaining ones received a combination consisting of BILN-2061 and mAb1671. During a six-week period BILN-2061 was administered (10 mg/kg, BID, oral gavage) with or without additional injections of mAb1671 (400 μg per injection) at day 0, 2, 4, 7, 10, 13, 16, 20, 23, 27, 30, 34, 37, 41.

Results: In this study, we observed that human-liver chimeric mice receiving monotherapy of the protease inhibitor BILN-2061 rapidly experience on-therapy virologic breakthrough. Deep sequencing analysis of the HCV protease domain confirmed the dominant manifestation of PI-RAVs upon viral rebound. In contrast, such on-treatment breakthrough was completely absent in mice treated with a combination of the PI with mAb1671. Our data suggest that mAb1671 precludes the emergence and dissemination of PI-RAVs during combination therapy.

Conclusions: We provide preclinical *in vivo* evidence that the addition of an entry inhibitor to a DAA regimen is an excellent strategy to prevent therapeutic failure due to the selection and rebound of DAA-RAVs.

G10

MITOCHONDRIAL-TARGETED ANTIOXIDANT MITOQUINONE REDUCES PORTAL HYPERTENSION IN CCl_4-CIRRHOTIC RATS BY DECREASING INTRAHEPATIC RESISTANCE

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Background and Aims: Increased intrahepatic resistance (IHR) due to both architectural alterations of the liver parenchyma as well as to dynamic increase in the hepatic vascular tone, is the primary factor in the development of portal hypertension. Hepatic Stellate Cells (HSC) are the main cells contributing to disrupt liver parenchyma by promoting fibrogenesis when activated. We have shown that cirrhotic livers have an increased production of reactive oxidative species (ROS), and that antioxidant therapy decreases portal pressure. It is also known that part of these ROS is produced within mitochondria. The aim of this study was to asses if the mitochondrial-directed antioxidant Mitoquinone was able to reduce oxidative stress, decrease intrahepatic fibrosis and attenuate portal hypertension.

Methods: *In vitro*: The expression of *col1*, *α-SMA* and *pdgfrb* (qPCR) was determined in human activated HSC (LX-2) in response to Mitoquinone (1 nM for 24 h; n = 3). Proliferation and viability were also assessed.

In vivo: CCl_4-cirrhotic Wistar rats were treated with Mitoquinone (MIT: 25 mg/kg/day p.o.; n = 9) or its vehicle, DecylTPP (Veh: 25 mg/kg/day; n = 9) for 2 weeks. Cellular and mitochondrial oxidative stress was assessed by DHE and MitoSox staining. Systemic and hepatic hemodynamics were determined *in vivo*. Liver fibrosis was determined by Sirius Red staining and HSC phenotype by gene expression of *col1*, *col1α2, pdgfrb, timp1, timp2* (qPCR) and α-SMA and desmin expression (IHC).

Results: *In vitro*: Mitoquinone induced a significant deactivation of HSC (*col1* [-67%], *α-SMA* [-65%] and *pdgfrb* [-48%]), and decreased proliferation (−43%). No effects on viability were observed.

In vivo: Mitoquinone markedly decreased mitochondrial (−34%) and cellular (−34%) oxidative stress. These effects were associated with a significant reduction in PP (Veh: 13.6±0.9 *vs* MIT: 10.9±0.6 mmHg; −20%) without affecting PBF resulting in a decrease in IHR (−34%). MAP was not significantly modified. Livers from Mitoquinone-treated rats showed significantly lower fibrosis (−36%) and expression of *col1*, *pdgfrb* and *timp2* (−68%, −54% and −31%, respectively) without significant changes in α-SMA and desmin. These data suggest Mitoquinone evokes a change in HSC to a more inactive form.

Conclusions: Mitoquinone significantly reduces mitochondrial ROS, PP and IHR, mostly by decreasing collagen deposition due to inactivation of HSC. We herein propose directed mitochondrial antioxidants as a novel treatment against portal hypertension and cirrhosis.

G11
MICROBIAL TRANSLOCATION IN LIVER FIBROSIS INDUCES CHRONIC IFNAR SIGNALING THAT IMPAIRS INNATE IMMUNITY AGAINST BACTERIAL INFECTION

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Background and Aims: A common clinical complication in patients with liver fibrosis or cirrhosis is enhanced susceptibility and failure to control bacterial infections.
Methods: To identify the underlying cellular and molecular mechanisms, we used the bile-duct ligation model for the induction of liver fibrosis in mice and characterized the immune responses to *Listeria monocytogenes* infections in the context of liver fibrosis and cirrhosis.
Results: Our data revealed an enhanced susceptibility to *Listeria* infections in mice with liver fibrosis/cirrhosis leading to persistence of infection, which recapitulates the clinical situations in humans with chronic liver disease. While the innate immunity and clearance of *Listeria* in the spleen of those mice was unaltered, the bactericidal activity of intrahepatic macrophages and bacterial clearance in the liver were strongly impaired. This reduced local clearance of *Listeria* correlates with impaired IFNγ, IL-12 and ROS production by myeloid cells in the liver.
Mechanistically, we identified IFNAR signaling in myeloid cells as initiating event for the impaired anti-bacterial immunity in mice with liver fibrosis. Using germ free mice we show that IFNAR signaling is induced by translocated gut microbiota during liver fibrosis. Chronic Type I IFN signaling in myeloid cells in turn causes IL-10 release, which directly inhibited IFNg, IL-12 and ROS production. Strikingly, we could rescue mice from chronic bacterial infection by either blocking IFN signaling specifically in myeloid cells or by systemic blockade of IL-10 signaling.
Conclusions: This murine model allowed to identify important IFNAR-mediated supressive mechanisms in chronic liver diseases, opening new therapeutics avenue to control bacterial infections in patients with liver fibrosis or cirrhosis.

G12
THE BURDEN OF CARDIOVASCULAR DISEASE AND MORTALITY ACROSS A SPECTRUM OF NON-ALCOHOLIC FATTY LIVER DISEASE: A 14-YEAR FOLLOW-UP POPULATION STUDY OF 929,465 INDIVIDUALS

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is recognised as a risk factor for cardiovascular disease (CVD) with some evidence, albeit from small studies, that non-alcoholic steatohepatitis (NASH) conveys greater risk than NAFLD. In this large UK study we studied the overall burden of CVD and all-cause mortality across the spectrum of NAFLD.
Methods: Anonymous data was obtained from a local computerised hospital activity analysis register regarding a total population of 929,465 patients in England area during 2000–2013. Data was processed using the ACALM (Algorithm for Co-morbidity, Associations, Length of stay and Mortality) study protocol, using ICD-10 codes to identify patients with NAFLD (K76.0), NASH (75.8), and NAFLD cirrhosis [cryptogenic cirrhosis (K74.6)] throughout the study period. Cardiovascular comorbidities were coded according to the ICD-10 criteria and their prevalence were analysed over 14-years.
Results: During the 14-year study period, 2701 patients were diagnosed with NAFLD-spectrum conditions: 1294 with NAFLD, 122 with NASH, and 1285 with cirrhosis. Mean ages at diagnosis were 51±0.4, 52±2 and 59±0.4 years, respectively. All groups had a male predominance (56–58%) and were 78–80% Caucasian. All-cause mortality was higher in NASH than NAFLD (22.1% vs. 14.5%, $p = 0.025$), and in cirrhosis than NAFLD (53.1% vs. 14.5%, $p < 0.001$). Congestive cardiac failure (CCF) was less prevalent in NAFLD than NASH ($p = 0.001$) and cirrhosis ($p < 0.001$). The prevalence of type 2 diabetes mellitus, atrial fibrillation, hyperlipidaemia, chronic kidney disease were higher in the advanced stages of NAFLD (Table 1). There was no difference in the prevalence of hypertension between the groups.

Table 1. Prevalence of mortality, metabolic and cardiovascular outcomes for patients within the spectrum of NAFLD during a 14-year study period

Clinical outcome	Number (%)		
	NAFLD (n = 1294)	NASH (n = =122)	Cirrhosis (n = 1285)
Congestive cardiac failure	49 (3.8)	11 (9.0)‡	85 (6.6)*
Atrial fibrillation	64 (4.9)	10 (8.2)	107 (8.3)**
Type 2 diabetes	271 (20.9)	30 (24.6)	401 (31.2)**
Chronic kidney disease	40 (3.1)	6 (4.9)	89 (6.9)**
Hyperlipidaemia	156 (12.1)	21 (17.2)	64 (5.0)**
Hypertension	381 (29.4)	42 (34.4)	351 (27.3)
Mortality	188 (14.5)	27 (22.1)†	682 (53.1)**

For NAFLD vs. NASH: †$p < 0.05$, ‡$p < 0.01$. For NAFLD vs. cirrhosis: *$p < 0.001$, **$p < 0.0001$.

Conclusions: By utilising a large population based cohort this study provides important new insights into mortality and cardiovascular disease in patients with NAFLD. Notably, during a 14-year study period, death occurs in over 20% of patients diagnosed with NASH and 50% with related-cirrhosis.

Liver tumours: Clinical

O048
FIRST SELECTIVE SMALL MOLECULE INHIBITOR OF FGFR4 FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMAS WITH AN ACTIVATED FGFR4 SIGNALING PATHWAY

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Introduction: Limited treatment options are available to patients with hepatocellular carcinoma (HCC) and the multi-kinase inhibitor sorafenib remains the only approved drug for this devastating disease. However, molecularly-stratified treatment schemes are beginning to be developed for HCC.
Material and Methods: Fibroblast growth factor 19 (FGF19), the ligand for fibroblast growth factor receptor 4 (FGFR4), is not expressed in normal liver, but is made in the ileum and delivered to the liver via portal circulation. However, a subset of human HCCs (7%) harbor amplification of *FGF19* and an additional one-fourth of tumors (21–34%) overexpress *FGF19* in the absence of gene amplification. In the presence of FGFR4 and its co-receptor *klotho β*

(*KLB*), *FGF19* overexpression can promote liver tumor formation in mice, a process which can be blocked by inhibiting FGFR4 signaling. This suggests that FGFR4 inhibition might be an effective treatment strategy in HCC patients whose tumors have an active FGF19/FGFR4 signaling axis.

Results: Here, we report the discovery of BLU9931, a potent and irreversible small molecule inhibitor of FGFR4. BLU9931 is exquisitely selective for FGFR4 versus other kinases, including FGFR family members. HCC cell lines with an activated FGFR4 signaling pathway were sensitive to FGFR4 inhibition and BLU9931 treatment decreased proliferation and induced apoptosis. BLU9931 also showed remarkable antitumor activity towards HCC tumor xenografts expressing *FGFR4*/*KLB* and harboring amplified *FGF19*. Furthermore, similar antitumor activity was observed in liver tumor xenografts which express detectable levels of *FGF19* mRNA and whose FGFR4 pathway remains intact, but lack *FGF19* amplification.

Conclusions: Taken together, these findings demonstrate a novel therapeutic strategy that targets a defined subset of HCC patients with a selective FGFR4 inhibitor and clinical trials to test this hypothesis are underway.

O049

SERUM LIPIDOMIC PROFILING FOR SCREENING POTENTIAL BIOMARKERS OF HEPATOCELLULAR CARCINOMA BY ULTRAPERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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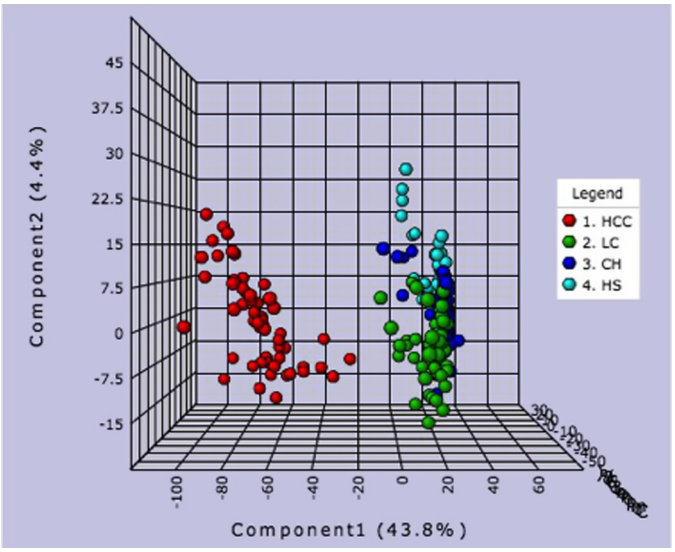
Background and Aims: Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and usually develops in patients with liver cirrhosis (LC). Biomarkers for HCC are limited. We explored the serum lipidome profiles of HCC to identify potential diagnostic biomarkers.

Methods: A total of 234 subjects were enrolled from 2011 to 2014. An ultraperformance liquid chromatography–mass spectrometry (UPLC-MS) lipidomic method was used to characterize serum profiles from HCC (n = 56), LC (n = 59), chronic hepatitis (CH) (n = 62), and healthy subjects (n = 57). Patients were diagnosed by laboratory and imaging evidence and all presented with hepatitis C virus-induced CH while healthy controls had normal liver function and no infectious diseases. Reversed-phased analysis was performed on a Waters ACQUITY IClass UPLC system coupled to a Waters Synapt-MS hidrid quadrupole-time of flight mass spectrometer in the positive ion electrospray mode with a mass scan range 200–1200 m/z for data acquisition in continuous mode. Data were processed with Progenesis software (Waters). The resulting multivariate data set was analyzed with MetaboAnalyst (TMIC) using supervised partial least-squares discriminate analysis (PLS-DA). Potential biomarkers were selected according to variable importance in the projection (VIP) and statistical significance was evaluated using Mann–Whitney test. The receiver operating characteristic (ROC) curve was performed to compare the accuracy of selected biomarkers and alpha-fetoprotein (AFP) levels in HCC diagnosis. To identify the potential biomarkers, the HMDB and LipidMaps databases were queried with the exact mass.

Results: The UPLC–MS-based serum lipidomic profile distinguished all HCC patients and provided more accurate diagnosis for LC patients than conventional AFP detection. HCC patients were discriminated from LC, CH and controls with 100% sensitivity and specificity. In comparison, AFP showed sensitivity and specificity of 18% and 98% for HCC diagnosis when the reference value was 200 ng/mL and 50% and 72%, respectively, in the range of 20 ng/mL. The 10 potential biomarkers comprised cardiolipins, glycerophospholipids and sphingolipids. Ganglioside GD3, an acidic glycosphingolipid involved in cell proliferation, differentiation and apoptosis, exhibited a significant increase in HCC and a decrease in LC and was proposed as an important indicator of HCC.

Conclusions: UPLC-MS lipid profiling proved to be an efficient and convenient tool for diagnosis and screening of HCC in a high-risk population.



O050

A NEW ALGORITHM FOR PREDICTING THE HEPATOCELLULAR CARCINOMA OCCURRENCE IN CIRRHOTIC PATIENTS

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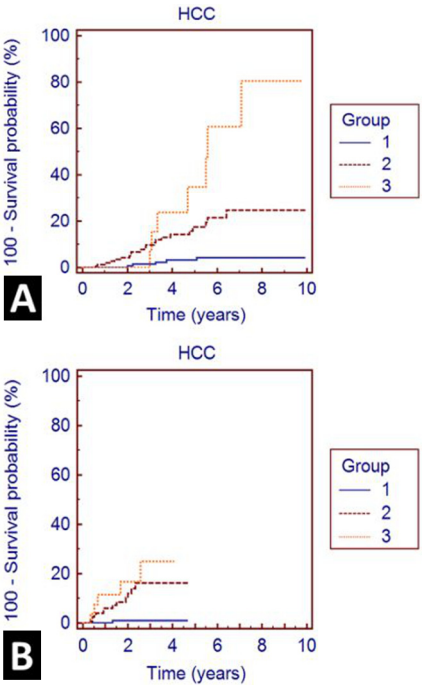
Background and Aims: Guidelines recommend ultrasound every 6 months for HCC screening in cirrhotic patients. However, due to the large number of patients/limited resources some biomarkers for prioritizing patients for screening would be helpful. We aimed to develop an algorithm for predicting HCC incidence in a cohort of mixed etiologies of cirrhosis.

Methods: We retrospectively collected data of cirrhotic patients evaluated between January 2004 and January 2014. In the same session hepatovenous pressure gradient (HVPG) measurements, laboratory parameters and tumor markers (AFP) were determined. Patients with HCC at first presentation and those with a follow-up shorter than 90 days were excluded. HCC diagnosis was established by imaging/biopsy during follow-up.

Results: We identified 940 cirrhotic patients who were evaluated with HVPG and laboratory blood tests. We excluded 167 patients with HCC at first presentation and 93 with follow-up <90 days. The remaining patients were divided in 2 groups: training cohort (379 patients: 2004–2009) and validation cohort (301 patients: 2010-January 2014). The HCC incidence in the training and validation cohort was: 8.7% [median follow-up 3.75 years (0.25–9.9 years)] and 6.3% [median follow-up: 1.75 years (0.25–4.7 years)].
Univariate analysis in the training cohort showed that presence of clinically significant portal hypertension (p = 0.007), platelet count <100,000/mm^3 (p < 0.0001), AFP >10 ng/ml (p = 0.002), esophageal varices (p = 0.03), and age >50 years (p = 0.04) were associated

with HCC occurrence. The following factors were not associated with HCC occurrence: gender, liver cirrhosis etiology, presence of diabetes mellitus, HIV coinfection, BMI, Child–Pugh class, MELD score, presence/history of ascites, presence/history of hepatic encephalopathy, aminotransferases values, GGT, alkaline phosphatase, bilirubin, albumin.
In multivariate analysis, platelet count <100,000/mm^3 (OR = 4.36), age >50 years (OR = 2.46) and AFP >10 ng/ml (OR = 2.36) reached statistical significance.
Based on the odds ratio, the following points were assigned: platelet count <100,000/mm^3 – 2 points, age >50 years – 1 point, and AFP >10 ng/ml – 1 point.
The patients were classified in three groups: group 1 0–1 points, group 2 2–3 points and group 3 4 points.
The HCC incidence/year in the three groups in the training and validation cohorts was 0.6%, 3.2% and 9.2% ($p < 0.0001$) (Fig. 1A) and respectively 0.3%, 5.5% and 9.2% ($p < 0.0001$) (Fig. 1B).



Conclusions: The proposed algorithm can identify the patients with very low, moderate and high risk of HCC, which could be used for more targeted screening approaches.

O051
SOX9 IS A NOVEL CANCER STEM CELL MARKER SURROGATED BY OSTEOPONTIN IN HUMAN HEPATOCELLULAR CARCINOMA

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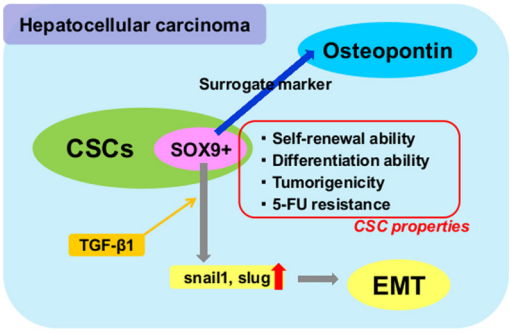
Background and Aims: Cancer stem cells (CSCs) are receiving considerable attention as targets for cancer therapy. Although sex determining region Y-box 9 (SOX9) is known to be an important marker in normal liver development, the relationship between SOX9 and CSCs of hepatocellular carcinoma (HCC) is unclear. Here, we aimed to determine if SOX9 can use as a new CSC marker in HCC.

Methods: We examined SOX9 expression of consecutive 166 human primary HCC specimens and 11 extrahepatic HCC metastatic tissues using immunohistochemistry, and analyzed the serum osteopontin level of SOX9$^+$/SOX9$^-$ patients. We transfected the SOX9 promoter-driven enhanced green fluorescence protein gene into HCC cell lines, and investigated fluorescence-activated cell sorting (FACS)-isolated SOX9$^+$/SOX9$^-$ cells.
Results: SOX9$^+$ HCC patients had significantly poorer recurrence-free survival, stronger venous invasion, and higher serum osteopontin level than SOX9$^-$ patients. SOX9 expression was strongly correlated with osteopontin expression in both primary HCC and metastatic HCC regions. Additionally, co-expression of SOX9 and osteopontin were detected more frequently in metastatic HCC regions than in primary HCC regions. FACS-isolated single SOX9$^+$ cells showed the ability to self-renew and differentiate into SOX9$^-$ cells, while single SOX9$^-$ cells never produced SOX9$^+$ cells. SOX9$^+$ cells displayed significantly greater proliferation capacity, higher sphere forming ability, and stronger 5-fluorouracil resistance based on higher expression of multidrug-resistant protein 5 in vitro. Xenotransplantation into immunodeficiency mice revealed that SOX9$^+$ cells could reproduce themselves, differentiate into SOX9$^-$ cells, and generate larger tumors at a higher frequency in vivo. SOX9$^+$ cells also showed greater motility and higher expression of EMT-related genes than SOX9$^-$ cells. Moreover, SOX9$^+$ cells showed higher osteopontin expression than SOX9$^-$ cells, and this property was suppressed by SOX9 knockdown via RNA interference.



Conclusions: SOX9$^+$ cells possess cancer stem cell properties correlated with EMT in human HCC. Osteopontin should be expected the clinical application as a useful surrogate marker of SOX9.

O052
ASSESSMENT OF THE HONG KONG LIVER CANCER STAGING SYSTEM IN EUROPE

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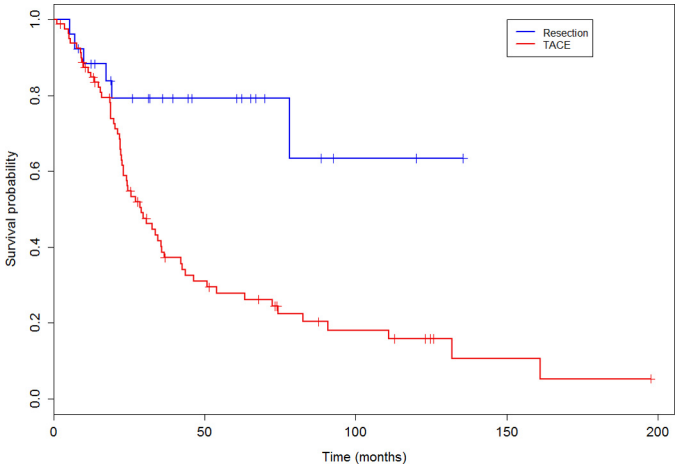
Background and Aims: European guidelines have endorsed the Barcelona Clinic Liver Cancer (BCLC) staging system. The aim of this study was to assess the performance of the recently developed Hong Kong Liver Cancer (HKLC) classification as a staging system for hepatocellular carcinoma (HCC) in Europe.
Methods: We used a pooled set of 1671 HCC patients combining three prospective European cohorts. Discrimination ability between the nine substages and five stages of the HKLC classification system was assessed. Cox regressions were used to evaluate the predictive

power of the HKLC and BCLC staging systems on overall survival (OS). The treatment algorithms were compared for patients with intermediate HCC, assessing the OS between surgical resection and trans-arterial chemoembolisation (TACE).
Results: The HKLC classification in nine substages shows suboptimal discrimination between the staging groups. The classification in five stages shows better discrimination between groups, with all stages being statistically significantly different. However, the BCLC classification performs better than the HKLC classification in the ability to predict OS. Comparing treatment options for patients with intermediate HCC (Figure), the survival of patients treated with surgical resection was better than the survival of patients treated with TACE (5-year survival probability: 79% vs 28%, p = 0.001).
Conclusions: The BCLC staging system performs better for European patients than the HKLC staging system. The superior survival of patients with intermediate HCC undergoing surgical resection is remarkable. This work highlights the need to identify criteria for selecting patients with intermediate HCC who benefit from surgical resection.



O053
GALNT14 GENOTYPE INDEPENDENTLY PREDICTS COMPLETE THERAPEUTIC RESPONSES OF TRANSCATHETER ARTERIAL CHEMOEMBOLIZATION IN PATIENTS WITH UNRESECTABLE HEPATOCELLULAR CARCINOMA

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Background and Aims: Recent prospective study indicated that genotype of polypeptide N-acetylgalactosaminyltransferase 14 (GALNT14) predicted chemotherapy responses as well as overall survivals in patients with advanced hepatocellular carcinoma (HCC). In the present study, we examined the predictive value of this marker in HCC patients receiving transcatheter arterial chemoembolization (TACE). The molecular mechanisms responsible for such correlations were also explored.
Methods: 327 HCC patients receiving TACE were included. Complete response was defined as absence of viable HCC tissues after TACE under computed tomography examination for at least 3 months. The GALNT14 genotype was determined by PCR – sequencing method. The predictive value was assessed by Cox proportional hazard model through calculation of time-to-complete response. 44 paired of cancerous and non-cancerous tissues derived from TACE-treated patients (previously receiving surgical resection) were submitted for phenotypic examination of GALNT14 expression levels.
Results: Of 327 TACE-treated HCC patients, 95 (29.0%) were rs9679162 "TT" and 232 (70.9%) were "non-TT" ("GG" + "GT") genotype. Univariate and multivariate Cox proportional hazard analysis including all clinical and biochemical factors showed that only previous ablation therapy (P = 0.017) and GALN14 "TT" genotype (P = 0.004) independently predicted favorable (shorter) time-to-complete therapeutic responses. Examination of the HCC tissues derived from TACE-treated patients showed that "TT" genotype was associated with a lower GALNT14 expression level, a higher cancerous/non-cancerous ratio of GALNT14 levels and a lower cancerous/non-cancerous ratio of cFLIP-S levels. Mass spectrum analysis showed that O-glycosylation of DR4/5 drastically increased in the cancerous tissue of "TT" genotype, compatible with a pro-apoptotic phenotype.
Conclusions: GALNT14 genotype independently predicted complete responses of TACE in HCC patients. Molecular analysis of the HCC tissues revealed that the GALNT14 "TT" genotype was associated with a pro-apoptotic phenotype.

O054
TM6SF2-T AND PNPLA3-G GENETIC VARIANTS CO-MODULATE THE RISK OF HEPATOCELLULAR CARCINOMA IN CAUCASIAN PATIENTS WITH ALCOHOLIC CIRRHOSIS. INTER-COHORT VALIDATION IN 1068 PATIENTS

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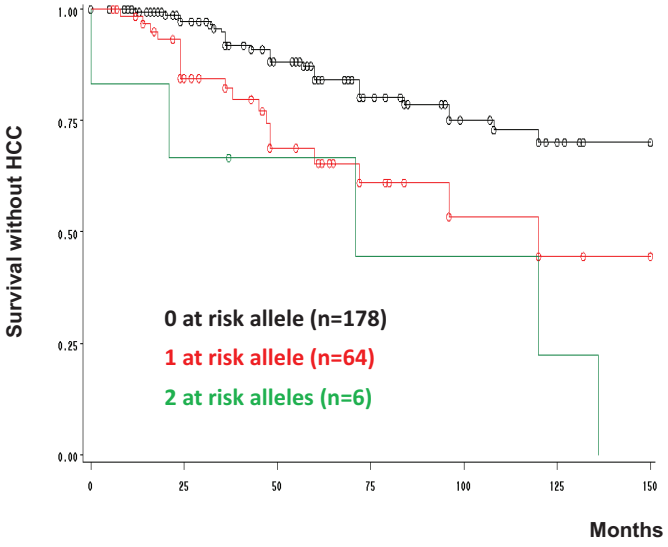
Background and Aims: A genetic variant, TM6SF2 (rs58542926) C>T, involved in lipid metabolism, has been recently linked to liver damage in patients with NAFLD. The aims of this study were (1) to assess the potential impact of TM6SF2 (rs58542926) C>T on alcoholic carcinogenesis, and (2) to evaluate its contribution to HCC risk stratification in combination with PNPLA3 (rs738409) C>G.
Methods: Three distinct cohorts were genotyped for both SNPs. The first one (Belgium, cohort 1/ALD) was a case-control cohort including alcoholic cirrhotics without (n = 484) and with HCC (n = 71). The second cohort (France, cohort 2/ALD) was a prospective cohort of alcoholic cirrhotics (n = 249), enrolled between 1997 and 2007, currently followed-up and screened for HCC (incident cases during 68 months follow-up, n = 51, 21%). The third cohort (France, cohort 3/HCV) was similar to the previous one and included 267 patients with HCV-related cirrhosis in whom 84 (31%) HCC were detected during 102 months of follow-up.
Results: In the case-control cohort 1/ALD, patients with HCC were highly enriched in TM6SF2-T risk allele [20 (28.2%) vs. 70 (14.5%), $P = 0.0006$]. Using a multivariable model (including age, gender, BMI and diabetes), this variant was independently associated with the presence of HCC [OR = 2.5 (1.4–4.3), $P = 0.002$]. In multivariable analysis, the risk for HCC increased in carriers of more than one PNPLA3-G and/or TM6SF2-T risk allele compare to those without (OR = 2.8 [1.3–5.7], P = 0.007). In the prospective cohort 2/ALD, patients with the TM6SF2-T allele had a higher risk of HCC occurrence (HR = 2.49 [1.5–4.5], P = 0.003). When the population was stratified into three groups according to the presence of none (n = 179), at least one (n = 64) or two (n = 6) PNPLA3-G and/or TM6SF2-T risk alleles, the number of HCC cases gradually increased [27 (15.1%) vs. 19 (29.7%) vs. 5 (83.3%) respectively, $P < 0.0001$]. This genotypic combination was an independent risk factor associated with HCC onset (HR = 2.31 [1.5–3.4], $P < 0.0001$). Conversely, in the

prospective cohort 3/HCV, TM6SF2-T allele was not significantly associated with HCC.

Conclusions: TM6SF2 (rs58542926) C>T is associated with HCC occurrence in patients with alcoholic cirrhosis but not with HCV infection. The risk of HCC increases in parallel with the number of PNPLA3-G and/or TM6SF2-T mutant alleles.



HR=2.31 [1.53-3.47], *P*<0.0001

O055

PUSHING THE LIMITS FOR TACE: SELECTION FOR TRANSARTERIAL CHEMOEMBOLISATION TREATMENT (STATE) SCORE IDENTIFIES HCC PATIENTS AT BCLC STAGE C SUITABLE FOR TACE

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Background and Aims: Recently, we developed the STATE score, which guides the decision for the first treatment with transarterial chemoembolization (TACE) in patients with intermediate stage hepatocellular carcinoma (HCC).

Here, we investigated the prognostic impact of the STATE-score in patients with advanced HCC (BCLC stage C).

Methods: 80 patients diagnosed with HCC at BCLC-stage C and treated with transarterial treatments between 1/2002 and 12/2011 at the Medical Universities of Vienna (training cohort, n = 61) and Innsbruck (validation cohort, n = 19) were included. Patients with major branch portal vein thrombosis were excluded.

The STATE-score is calculated by using the serum-albumin level (mg/dl), which is reduced by 12 points each, if the tumor load exceeds the up-to-7 criteria and/or CRP-levels are ≥1 mg/dl (maximum reduction: 24 points).

The up-to-7 criteria (in vs. out) were used to assess intrahepatic tumor load only, while macrovascular invasion, lymph-node and distant metastases were separately included into statistical analysis.

Results: The STATE score discriminated two groups [<18 (n = 36) vs. ≥18 points (n = 44)] with different prognosis (median OS: 5.2 vs. 14.3 months, p = 0.001): and remained a valid predictor of OS independent of Child–Pugh score, ECOG-PS, macrovascular invasion, lymph-node, distant metastasis and subsequent sorafenib treatment.

These results were confirmed upon separate analysis of patients treated in the training and the validation cohort.

Conclusions: The STATE-score identifies patients with advanced HCC who may be suitable for TACE.

Viral hepatitis C: Clinical

O056

LEDIPASVIR/SOFOSBUVIR TREATMENT RESULTS IN HIGH SVR RATES IN PATIENTS WITH CHRONIC GENOTYPE 4 AND 5 HCV INFECTION

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Background and Aims: Hepatitis C virus (HCV) genotype (GT) 4 is estimated to account for 8% to 13% and GT5 for ~1% of all chronic HCV infections globally (Gower et al. 2014 J Hepatol;61:S45-S57; Messina et al. 2014 Hepatology doi: 10.1002/hep.27259). GT4 HCV is found primarily in the Middle East and Sub-Saharan Africa and GT5 HCV primarily in Southern Africa. Clinical studies evaluating the treatment outcome with new direct acting antiviral agents in GT4 and especially GT5 HCV infection have been limited. The aim of the current ongoing study is to assess the safety, tolerability and efficacy of ledipasvir/sofosbuvir (LDV/SOF) in patients with chronic GT4 or GT5 HCV infection.

Methods: Treatment-naïve and treatment-experienced patients with chronic GT4 or GT5 HCV infection were enrolled at 5 sites in France to receive 12 weeks of LDV/SOF (90 mg/400 mg daily). Up to 50% of patients could have compensated cirrhosis at screening. The primary endpoint is the SVR12 rate with SVR4 as a secondary endpoint.

Results: 85 patients were enrolled: 44 patients had GT4 and 41 patients had GT5 chronic HCV infection. Overall, GT4 patients were 64% male, mean age 51 years, 50% were treatment-experienced, 18% had IL28B-CC genotype, 23% had compensated cirrhosis, and 70% had HCV RNA ≥800,000 IU/mL at baseline. Overall, GT5 patients were 51% male, mean age 63 years, 49% were treatment-experienced, 46% had IL28B CC genotype, 22% had compensated cirrhosis, and 85% had HCV RNA ≥800,000 IU/mL at baseline.

SVR4 rates are shown in the Table. Complete SVR12 data will be presented.

Table: SVR4 rates following LDV/SOF in GT4 and GT5 HCV infection

	SVR4 rate, % (n/N)	
	GT4	GT5
Overall	93 (41/44)	93 (38/41)[a]
Treatment naive	95 (21/22)	90 (19/21)[a]
Treatment experienced	91 (20/22)	95 (19/20)
Cirrhosis	100 (10/10)	89 (8/9)
No cirrhosis	91 (31/34)	94 (30/32)

[a] 1 patient lost to follow-up.

The most common adverse events (>10% of patients) were asthenia, headache, and fatigue. Most AEs were mild or moderate in severity and none resulted in treatment discontinuation. One patient

experienced an SAE of worsening of pre-existing depression which was considered unrelated to study drug. There were no grade 3 or 4 clinical laboratory abnormalities.
Conclusions: A ribavirin- and interferon-free regimen of ledipasvir/sofosbuvir administered in a fixed-dose combination tablet once daily for 12 weeks resulted in high SVR4 rates of 93% in GT4 and in GT5 HCV-infected treatment-naïve and treatment-experienced patients with or without cirrhosis. This regimen was well tolerated and represents a simple, highly effective all-oral treatment option which could facilitate more widespread treatment of patients.

O057

LONG-TERM FOLLOW-UP OF TREATMENT-EMERGENT RESISTANCE-ASSOCIATED VARIANTS IN NS3, NS5A AND NS5B WITH PARITAPREVIR/R-, OMBITASVIR- AND DASABUVIR-BASED REGIMENS

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Background and Aims: Approximately 3000 HCV genotype (GT) 1-infected patients have been treated with paritaprevir (formerly ABT-450, identified by AbbVie and Enanta)/ritonavir (designated paritaprevir/r), ombitasvir (formerly ABT-267), and/or dasabuvir (formerly ABT-333) +/− ribavirin (RBV) in three Phase 2 (M12–746, M12–998 and AVIATOR) and six Phase 3 (Sapphire-I, Sapphire-II, Pearl-II, Pearl-III, Pearl-IV, and Turquoise-II) clinical trials. Patients with treatment-emergent resistance-associated variants (RAVs) in these studies are being followed to post-treatment week (PTW) 48 for evaluation of the persistence of RAVs.
Methods: The persistence of treatment-emergent RAVs in HCV NS3, NS5A and NS5B was assessed in patients who did not achieve SVR in Phase 2 and 3 clinical trials, with available data through at least PTW24, by population and clonal sequencing.
Results: In patients who experienced virologic failure in the Phase 2 and 3 clinical trials, the predominant RAVs in GT1a were R155K and D168V in NS3, M28T and Q30R in NS5A, and S556G in NS5B. For paritaprevir, RAVs (any position) in NS3 persisted at detectable levels through at least PTW24 in 45% (31/69) of the patients, and through PTW48 in 7% (4/55) of patients. NS3 R155K remained detectable in 66% (8/12) and 25% (2/8) of the patients through PTW24 and 48, respectively; and D168 variants remained detectable in 37% (21/56) and 2% (1/53) of the patients through PTW24 and 48, respectively. For ombitasvir, RAVs in NS5A including M28T and Q30R persisted at detectable levels through PTW24 in 98% (67/68) of the patients, and through PTW48 in 96% (50/52) of the patients. For dasabuvir, RAVs in NS5B persisted at detectable levels through at least PTW24 in 75% (30/40) of the patients, and through PTW48 in 61% (22/36) of the patients. NS5B S556G persisted in 89% (24/27) and 79% (19/24) of patients through PTW24 and 48, respectively. Minor variants G558R and D559G/N in NS5B were not detectable at PTW24 and 48. Due to very high SVR rates in GT1b, trends in persistence of treatment-emergent RAVs could not be evaluated.
Conclusions: Studies to evaluate persistence of treatment-emergent RAVs in paritaprevir/r, ombitasvir and dasabuvir-based regimens are ongoing. In GT1a, the proportion of subjects with RAVs in NS3 declined over time, while most subjects with RAVs in NS5A and NS5B had these variants detectable through Post-Treatment Week 48.

O058

INCREASED CANCER RATES IN PATIENTS WITH CHRONIC HEPATITIS C: AN ANALYSIS OF THE CANCER REGISTRY IN A LARGE U.S. HEALTH MAINTENANCE ORGANIZATION

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Background and Aims: Hepatitis C virus (HCV) is an oncogenic virus, and an increased risk of malignancy in HCV has previously been reported. Cancer types associated with HCV include non-Hodgkin's lymphoma, renal and prostate cancers; as well as liver cancer. The aim of this study was to describe cancer rates in our cohort of HCV patients compared to cancer rates in the non-HCV population.
Methods: This is a retrospective study at Kaiser Permanente Southern California (KPSC), a large health maintenance organization with 3.5–4 million members. The KPSC cancer registry is an accredited program maintaining a complete profile of all cancer diagnoses for all KP members. In this study, we recorded all cancer diagnoses in patients ≥18 years of age with or without HCV during 2008–2012.
Results: From 2008 to 2012, 145,210 patient years were included in the HCV cohort, and 13,948,826 patient years were included in the non HCV cohort. Mean age at cancer diagnosis in the HCV cohort was 61.8 years, in the non-HCV cohort, 63.5 years. In the HCV cohort there were 2,213 cancer diagnoses (1524/100000) during the 5 year period and 1,654 cancer diagnoses when liver cancer was excluded (1139/100000). In the non HCV cohort there were 84,419 cancer diagnoses (605/100000) during the same 5 year period and 83,795 (601/100000) when liver cancer was excluded. The rate ratios between the HCV and non HCV cohorts for the total number of cancer cases including and excluding liver cancer are shown in Table 1.
Conclusions: In our cohort of Hepatitis C infected patients, cancer rates were significantly increased compared to the non-HCV cohort. This suggests that another extrahepatic manifestation of HCV may be an increased risk of cancer.

Table 1.

Cancer diagnosis 2008–2012	Rate			
Site	HCV members N = 145,210	Non HCV members N = 13,948,826	Rate ratio, HCV vs Non HCV	p-value
Head & neck	47.5	16.7	2.85 (2.24, 3.62)	<0.0001
Esophagus	10.3	4.4	2.34 (1.40, 3.91)	0.0011
Stomach	28.2	9.1	3.10 (2.27, 4.23)	<0.0001
Colon/rectum	106.7	55.2	1.93 (1.65, 2.27)	<0.0001
Liver	385.0	4.5	86.05 (76.77, 96.46)	<0.0001
Pancreas	34.4	12.6	2.74 (2.07, 3.63)	<0.0001
Lung/bronchus	111.6	46.8	2.38 (2.04, 2.79)	<0.0001
Prostate	174.9	88.4	1.98 (1.75, 2.24)	<0.0001
Renal	66.8	20.4	3.27 (2.67, 4.00)	<0.0001
Non-Hodgkin's lymphoma	80.6	22.2	3.63 (3.02, 4.37)	<0.0001
Myeloma	22.7	7.8	2.93 (2.07, 4.14)	<0.0001
All sites, incl liver cancer	1524	605	2.52 (2.41, 2.63)	<0.0001
All sites, excl liver cancer	1139	601	1.90 (1.81, 1.99)	<0.0001

O059

LONG-TERM PERSISTENCE OF HCV NS5A VARIANTS AFTER TREATMENT WITH NS5A INHIBITOR LEDIPASVIR

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Background and Aims: Ledipasvir (LDV) is a hepatitis C virus (HCV) NS5A inhibitor with potent antiviral activity against HCV genotype 1a and 1b. The aim of this study is to evaluate the persistence of resistance-associated variants (RAVs) in subjects who did not achieve SVR in previous Gilead-sponsored HCV clinical studies after receiving LDV without SOF who were then enrolled in a 3-year follow-up registry study.
Methods: Specimens from subjects treated with LDV in Gilead studies GS-US-248-0120, GS-US-248-0121, GS-US-248-0131, GS-US-248-0132, GS-US-256-0124 and GS-US-256-0148, were analyzed to determine their NS5A RAVs at baseline (BL) in the parent study (n = 84), post-BL in the parent study (n = 78), BL in the registry study (n = 80), and follow-up (FU) visits weeks 12 (n = 66), 24 (n = 66), 36 (n = 19), 48 (n = 80) and 96 (n = 65) in the registry study. Population sequencing was used for majority of samples at BL and Post-BL of the parent study, and deep sequencing for all samples in the registry study. If RAVs were observed, samples from subsequent timepoints were deep sequenced until RAVs were no longer detected (1% detection threshold).
Results: Of the 84 subjects with sequencing at BL in the parent study, 12 had NS5A RAVs at BL that persisted through FU96 except A92T that wasn't observed at Post-BL. NS5A RAVs were detected in 95% of subjects at virologic failure in the parent study and in 97%, 96.5%, 100%, 100%, 96%, and 94.0% of the subjects at BL, FU12, FU24, FU36, FU48 and FU96 visits in the registry study, respectively. At BL in the registry study subjects had up to 9 NS5A RAVs compared to FU96 when most subjects had <5 RAVs except 2 subjects with 7 RAVs. Except L31M, all variants: K24N/R, M28T/A, Q30E/H/K/R, L31V, H58D and Y93H/N/C were detected in fewer subjects at FU96 than BL. While the frequencies of some variants declined slowly over time in some subjects, at FU96, 51 of 54 (94.4%, 48 GT 1a and 3 GT 1b) subjects had NS5A RAVs detected by deep sequencing. For the 3 GT 1b subjects, the observed variants were L31I/M/V and Y93H/T and had frequencies up to 99.7% of the viral population. In GT1a subjects, most subjects had Q30R, L31M and H58D. Highest frequencies of variants were seen for K24N (73.2%), K24G (63.0%), H58D (57.2%), Y93C (56.9%), L31M (48.3%), Q30R (45.1%), Q30H (33.8%) and Y92N (27.6%).
Conclusions: NS5A variants can persist for >96 weeks post-treatment in subjects who relapse to regimens containing NS5A inhibitor suggesting high fitness of the NS5A RAVs.

O060

MIR-17/92 EXPRESSION PATTERN: A MOLECULAR SIGNATURE OF HCV-RELATED MIXED CRYOGLOBULINEMIA

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Background and Aims: HCV chronic infection is closely related to the development of lymphoproliferative disorders (LPDs), mainly mixed cryoglobulinemia (MC) and some subtypes of B-cell lymphoma. The pathogenesis of HCV-LPDs is still largely unknown. Modification of the expression levels of specific microRNAs (miRNAs) has been associated with different autoimmune and/or LPDs. In particular, the endogenous miR-17/92 cluster is very often amplified in cancer and in autoimmunity. Scarce data exist about the modifications of miRNA expression levels in HCV-related LPDs.
The aim of this study was to evaluate the modifications of the expression levels of the miR-17/92 cluster in a large population of HCV patients with and without MC and the effects of viral eradication.
Methods: The expression levels of miR-17/92 cluster were evaluated by Real Time PCR in PBMC samples from 79 HCV patients: 34 without LPD [HCV] and 45 with MC [HCV-MC]; among the 45 MC patients were included 20 patients who experienced a sustained virological and clinical response after antiviral treatment and of which pre and post therapy sampling was available. Relative expression levels of all the members of the miR-17/92 cluster (namely, miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a) were evaluated with the 2-ΔΔCt method, using miR-let-7d as housekeeping.
Results: All the members of the miR-17/92 cluster were highly upregulated in PBMCs from HCV-MC patients ($p < 0.001$) when compared to HCV group. A restoration of miRNAs levels was observed in the samples taken after viral eradication (miR-17, miR-18a, miR-19a, miR-19b, miR-20a, $p < 0.001$ and miR-92a, $p < 0.05$, when compared with pre-treatment levels). Regarding miR-20a, the levels in samples taken after HCV eradication continued to be significantly higher than in controls (HCV patients) ($p < 0.05$), in spite of the sudden fall observed after therapy.
Conclusions: This study, for the first time, shows that the expression pattern of miRNA-17/92 cluster is modified in PBMC from HCV patients with MC. The sudden restoration of these values of expression in patients achieving a sustained virological and clinical response after antiviral treatment, strongly suggests that miR-17/92 cluster plays a key role in the pathogenesis of MC.

O061

INCIDENCE OF HEPATITIS C REINFECTION FOLLOWING SUSTAINED VIROLOGICAL RESPONSE – A SEVEN YEAR FOLLOW-UP OF SCANDINAVIAN PATIENTS INFECTED THROUGH INJECTING DRUG USE

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Background and Aims: Access to hepatitis C (HCV) treatment among people who inject drugs (PWID) remains limited and is often withheld based on the perceived risk of reinfection following treatment. No prospective study has yet given any robust estimate of this risk, an issue that will become increasingly important in the interferon-free era. The aim of this study was therefore to assess the long-term incidence of HCV reinfection in PWID following sustained virological response (SVR).
Methods: In 2004–2005 we performed a multicentre treatment study comprising 428 monoinfected HCV genotype 2 or 3 patients in the Scandinavian countries Norway, Sweden and Denmark (the North-C trial). The overall SVR rate was 76% and two thirds were PWID who had all been abstinent from drug use at least six months prior to treatment. This follow-up study was performed in 2012–14 and all Norwegian patients (22 study sites) who had obtained SVR (n = 161) were eligible for participation. Clinical, laboratory and behavioural data were collected and the incidence of HCV reinfection was calculated using person-time techniques.
Results: Follow-up data were available in 137/161 (85%) individuals (60% male, median age 44 years) with a median follow-up time of 7.1 years (IQR 1.5). Of 92 (67%) individuals who had acquired HCV infection through injecting drug use [follow-up time 610 person-years (PY)], 36 (40%) had relapsed to injecting behaviour after treatment (follow-up time 232 PY). Recurrence of HCV RNA

was identified in 12 patients and 11 of those cases occurred in individuals who had relapsed to injecting drug use. The incidence of reinfection was 1.8/100 PY (95% CI 1.0–3.2) among individuals with injecting drug use prior to treatment, 4.7/100 PY (95% CI 2.7–8.4) among those who had relapsed to injecting behaviour during follow-up and 0.32/100 PY (95% CI 0.04–2.2) among those with no history of injecting drug use. In multivariate regression analysis, low education level (OR 7.3, 95% CI 1.8–29.7; p = 0.006) was the only independent baseline predictor for HCV reinfection.

Conclusions: In this seven year follow-up study mainly comprising PWID who had been abstinent from drug use at least six months prior to HCV treatment, we demonstrate frequent relapse to injecting drug use but still low to moderate long-term incidence of HCV reinfection (1.8/100 PY). Our findings are in line with estimates from previous smaller studies and should be addressed when providing HCV care for PWID.

O062

FACTORS ASSOCIATED WITH SPONTANEOUS CLEARANCE OF CHRONIC HEPATITIS C VIRUS INFECTION: A RETROSPECTIVE CASE CONTROL STUDY

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Background and Aims: Chronic HCV infection (CHC) develops in ~75% of people who acquire HCV, and is defined as infection persisting beyond six months. Spontaneous clearance of CHC is rare and poorly characterised. We conducted a retrospective case control study of patients attending centres in Glasgow to identify factors associated with spontaneous clearance of CHC.

Methods: Data were obtained from the West of Scotland specialist virus laboratories on HCV testing between 1994 and 2013. Patients with ≥2 sequential RNA positive samples at least 6 months apart, followed by at least one negative HCV RNA (potential spontaneous clearance cohort), were identified in addition to patients with ≥2 positive samples at least 6 months apart with no subsequent negative samples (chronic infection cohort). Both cohorts were linked to the Scottish Hepatitis C database to exclude treated patients and obtain relevant patient data. A control group was randomly selected from the chronic infection cohort with a frequency of 4 per spontaneous clearer. Case notes were reviewed to verify the clinical course. Categorical variables were compared using χ^2. Continuous variables were analysed using the exact Wilcoxon Mann–Whitney test. P values are 2-sided, and values of <0.05 were considered significant.

Results: 25113 HCV RNA positive samples, relating to 10318 patients were identified. 116 patients, not linked as treated, had 2 sequential positive results followed by a negative. 66 patients were excluded following case note review leaving 50 patients of interest, contributing 241 person years follow up. 2518 untreated, chronically infected patients were identified, contributing 13766 person years follow up. 200 control patients were randomly selected from this cohort. Table 1 summarises demographic and clinical characteristics of the study populations. Spontaneous clearance of CHC was associated with female gender, HBsAg positivity, younger age at infection and lower HCV RNA. Spontaneous clearance was negatively associated with current IDU. The overall incidence rate of spontaneous clearance of CHC was 0.36 per 100 person years follow up, occurring after a median 49.5 months of infection.

Table 1. Main demographic and clinical patient characteristics

	Late spontaneous clearance (n = 50)	Chronically infected (n = 200)	P value
Male sex [n (%)]	19 (38)	129 (64.5)	**0.001**
Median age [years (IQR)]	41 (37–48)	43 (38–49)	0.36
Median age at infection [years (IQR)]	28.5 (25–36)	33 (28–38)	**0.02**
Ethnic group [n (%)]			0.72
White	48 (96)	194 (97)	
Asian	2 (4)	6 (3)	
Risk group [n (%)]			0.72
Intravenous drug use	41 (82)	161 (80.5)	
Sexual/partner	2 (4)	3 (1.5)	
Iatrogenic	2 (4)	6 (3)	
Vertical	0 (0)	3 (1.5)	
Born abroad	1 (2)	5 (2.5)	
Unknown	4 (8)	22 (11)	
HCV genotype [n (%)]			0.71
1	7 (14)	61 (30.5)	
2	1 (2)	5 (2.5)	
3	9 (18)	52 (26)	
Unknown	33 (66)	82 (41)	
Serum HIV IgG [n (%)]			0.52
Positive	2 (4)	3 (1.5)	
Negative	36 (72)	98 (49)	
Not tested	12 (24)	99 (49.5)	
Serum HBsAg [n (%)]			**0.001**
Positive	5 (10)	0 (0)	
Negative	43 (86)	99 (49.5)	
Not tested	2 (4)	101 (50.5)	
Current IDU [n (%)]			**0.03**
Yes	15 (30)	97 (48.5)	
No	25 (50)	76 (38)	
Unknown	10 (20)	27 (13.5)	
History of alcohol excess/ALD [n (%)]			0.24
Yes	21 (42)	64 (32)	
No	24 (48)	109 (54.5)	
Unknown	5 (10)	27 (13.5)	
Cirrhosis [n (%)]			0.24
Yes	13 (26)	34 (17)	
No	25 (50)	104 (52)	
Unknown	12 (24)	62 (31)	
Median duration of infection [months (IQR)]	49.5 (30.5–81.25)	50 (19–101.5)	0.94
HCV VL (IU/ml)			**<0.001**
Median	1000 †	341,142	
Interquartile range	1000–375,792	59,496–1,517,864	

† Data on HCV VL only available for 20 patients.

Conclusions: This is the largest cohort of patients with spontaneous clearance of CHC studied to date. Our data suggest that clearance occurs rarely in CHC but can occur after a prolonged duration, and is associated with female sex, HBV co-infection, younger age at infection and low HCV RNA titres.

O063

HCV REINFECTION CASES IN PHASE 3 STUDIES OF SOFOSBUVIR

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Background and Aims: Relapse accounted for all virologic failures among treatment-adherent patients in the ledipasvir/sofosbuvir (LDV/SOF) and SOF Phase 3 clinical development program with most of relapse cases occurring 4–12 weeks post-treatment. It has been shown that SVR12 is an appropriate time point for the reliable assessment of a durable treatment response to SOF-containing regimens. However, in very rare cases, patients became positive again for HCV RNA after week 12 post-treatment. In this study, relapse cases that occurred after week 12 post-treatment in the phase 3 SOF studies were investigated for possible HCV reinfection.

Methods: Sequencing analysis of the NS5B gene was performed from patients who had relapse HCV after week 12 post-treatment in NEUTRINO, FISSION, POSITRON, FUSION, VALENCE, PHOTON-1, PHOTON-2 and GS-US-334-0118 SOF studies. Maximum likelihood

phylogenetic trees were constructed for baseline and relapse time points to investigate the genetic distance of these HCV samples. Longitudinal samples from patients with viral relapse prior to week 12 and then subsequent follow-up samples were used as a control for viral genetic drift over the same time period.
Results: In the 8 SOF studies, of 1154 patients who achieved SVR12, 1142 also had SVR24. However, there were 12 patients who achieved SVR12, but did not achieve SVR24 and had available samples for testing. Of 12 patients, samples from 10 patients were successfully sequenced at baseline and relapse and samples from 2 patients failed amplification at relapse. Phylogenetic analyses that included baseline and relapse samples from these 10 patients as well 30 patients that who relapsed prior to week 12 post-treatment and had longitudinal samples beyond week 12 post-treatment were performed. The genetic distance in NS5B between the baseline and relapse time points in 4 of 10 late virologic failures suggested a re-infection event and not relapse growth of the baseline HCV. In 1 of 4 reinfected patients, there was also a switch in genotype of virus between baseline and reinfection. Longitudinal samples from control patients showed minimal genetic drift over the same time period.
Conclusions: About half of the relapse cases in the SOF Phase 3 clinical development program who relapsed after achieving SVR12 were found to have HCV reinfection and not a relapse of the baseline virus. This result further supports SVR12 as an appropriate time point for the reliable assessment of a durable treatment response to SOF regimens.

Fatty liver disease: Experimental

O064
GUT–LIVER AXIS DERANGEMENT DUE TO LACK OF INFLAMMASOME ACTIVITY LEADS TO VISCERAL OBESITY AND NASH DEVELOPMENT

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Background and Aims: Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common form of chronic liver disease and can lead to cirrhosis and hepatocellular carcinoma. Gut microflora alterations and bacterial translocation induced by specific dietary habits can elicit a proinflammatory and profibrogenic response. The NLRP3 inflammasome regulates intestinal homeostasis and mediates the release of IL1β and IL18 in response to cellular danger signals. Its role in NAFLD development is controversial. Thus aim of the study was to investigate the role of NLRP3 inflammasome in a Western lifestyle diet model of NAFLD.
Methods: Wild-type (WT) C57BL/6 and Nlrp3A350VneoR ($Nlrp3^{-/-}$) mice were fed either a chow diet (controls) or a high-fat diet with fructose in drinking water (HFHC) for 12 weeks.
Results: $Nlrp3^{-/-}$ HFHC gained more weight ($p < 0.01$), showed reduced energy expenditure and more fat mass (both measured by doubly labeled water, all $p < 0.05$) with increased adipose tissue TNFα expression ($p < 0.05$), and developed more hepatic steatosis measured by triglyceride content ($p < 0.01$) compared to WT HFHC. HFHC increased intestinal permeability, as showed by reduced zonulin-1 expression in the caecum, that led to higher hepatic expression of TLR4 ($p < 0.01$) and TLR9 ($p < 0.05$) in $Nlrp3^{-/-}$ HFHC compared to WT HFHC. In the liver no differences were observed between $Nlrp3^{-/-}$ HFHC and WT HFHC in the expression of downstream SREBP-1c effectors of de novo lipogenesis (ACC, FAS and SCD-1) that were significantly increased in both groups. On the other hand, compared to WT HFHC, $Nlrp3^{-/-}$ HFHC showed higher expression of the lipogenic transcription factor PPARγ2 ($p < 0.01$) and of its downstream effectors FABP-4 ($p < 0.05$) and CD36 ($p < 0.01$) that regulate lipid uptake and storage. In addition $Nlrp3^{-/-}$ HFHC showed increased mitochondrial-oxidation of fatty acid (i.e., higher CPT1A expression) that, associated to the reduced expression of the "master regulator" of the antioxidant response NRF2, led to increased superoxide production (measured by dihydroethidium staining) (all $p < 0.01$). These series of events were associated to increased macrophage infiltration, type I collagen and MCP1 gene expression ($p < 0.05$) in $Nlrp3^{-/-}$ HFHC mice only.
Conclusions: In the Western lifestyle diet, lack of NLRP3 inflammasome is associated with translocation of bacterial products that leads to severe metabolic alterations in both adipose tissue and the liver, and to NASH development.

O065
MECHANISTIC STUDY OF TM6SF2 IN NAFLD PATHOGENESIS: STABLY TRANSFECTED Huh7 CELLS OVER-EXPRESSING THE TM6SF2 E167K VARIANT EXHIBIT GREATER LEVELS OF OXIDATIVE STRESS

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Background and Aims: Non-Alcoholic Fatty Liver Disease (NAFLD) is a complex disease trait influenced by genetic modifiers. We, and others, have recently reported that minor (T) allele carriage of a non-synonymous polymorphism in *TM6SF2* (rs58542926 C>T, p.E167K), a gene of unknown function, is associated not only with steatosis, but also with NASH and clinically relevant advanced liver fibrosis. Carriage of the C allele is associated with dyslipidaemia and cardiovascular disease. To better understand the mechanisms underlying these associations, we examined the effects of *TM6SF2* over-expression in vitro.
Methods: Huh7 human hepatoma cell-lines stably transfected to overexpress wild-type (E167) or variant (167K) forms of TM6SF2 were generated. Cells were cultured in standard medium or oleic acid enriched media. Hepatocyte lipid accumulation was assessed by oil red O staining, cellular oxidative stress (ROS) measured by FACS after DCFDA staining and the expression profile of relevant genes involved in lipid metabolism, fatty acid oxidation, inflammation and fibrogenesis were measured by rtPCR.
Results: Following stable transfection, *TM6SF2* expression was >50-fold increased relative to resting Huh7 cells both at mRNA and protein levels. As previously noted, protein expression of the E167 form was approximately twice that of the 167K variant, likely reflecting protein misfolding or accelerated intracellular degradation. Whilst overexpression of either *TM6SF2* form was associated with increased hepatic lipid accumulation in oleic acid enriched media, oil red O staining was substantially greater in cells expressing 167K. Consistent with development of NASH, carriage of the 167K variant was also associated with greater levels of ROS. At the transcriptome level, overexpression of *TM6SF2* suppressed several lipogenic and pro-inflammatory genes. However, relative to E167, the 167K variant promoted differential expression of key genes including *DGAT1, CYP2E1, IL6* and *TNFA*.
Conclusions: These in vitro data suggest that *TM6SF2* has profound effects on hepatocyte lipid metabolism. They provide the first evidence that the TM6SF2 p.E167K variant induces increased hepatocyte oxidative stress, possibly by favoring extra-mitochondrial omega- fatty-acid oxidation, and also promotes hepatocyte inflammatory cytokine expression. Work to further characterize the functional effects of TM6SF2 p.E167K carriage and the mechanisms through which it promotes liver injury and NASH are underway.

O066

Bcl-3 REGULATES HEPATIC GLUCOSE AND LIPID METABOLISMS THROUGH INSULIN AND ASSOCIATED METABOLIC TRANSCRIPTION FACTORS

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Background and Aims: The NFkB-cofactor gene B cell leukemia-3 (Bcl-3) plays a critical role in altering the transcriptional capacity of the NFkB-subunits p50 and 52 and thus regulates inflammation. Beyond that Bcl-3 was also identified as a transcriptional coregulator of genes involved in cellular energy metabolism. However, its regulatory role in non-alcoholic fatty liver disease (NAFLD) has not been investigated yet.
Methods: 8–12 week old male mice exhibiting a hepatocyte-specific overexpression of Bcl-3 (Bcl-3hepar) and wild type (wt) littermates were fed with a high-fat diet [HFD; 35.5% w/w crude fat (58kJ%)] plus drinking water enriched with fructose (55% w/v) and glucose (45% w/v) for 12 weeks to induce NAFLD. Gender- and age-matched mice received a corresponding control diet [CD; 5.4% w/w crude fat (13kJ%)] and normal drinking water. After the dietary period, biometric, serological, histological, FACS, Western Blot and qRT-PCR analyses were performed.
Results: HFD-feeding induced a pronounced metabolic derangement in Bcl-3hepar mice which was characterized by enhanced hepatic cell death and steatosis from increased de novo lipogenesis (ACC, FAS, SREBP-1), and decreased b-oxidation (CPT1, PPARa), hydrolysis (FXR, CES1) and release (MTTP) of fatty acids. In addition, Bcl-3hepar mice exhibited hyperinsulinaemia, but still a decreased expression of gluconeogenetic enzymes. Interestingly, the transcription factors PGC-1a, PPARa and PPARg, that regulate hepatic glucose and lipid metabolism and also promote anti-inflammatory processes, were down-regulated in the liver of HFD-fed Bcl-3hepar mice as compared to the wt. Ex vivo-treatment of primary hepatocytes from Bcl-3hepar mice with oleic acid, fructose and glucose for 48 h to induce intracellular lipid accumulation led to a diminished expression of PGC-1a, PPARa and PPARg, whereas these genes were not affected in wt hepatocytes. Moreover, metformin improves the metabolic and injurious phenotype of Bcl-3hepar mice in vivo by reducing hepatic steatosis via a decrease in lipogenesis (FAS) and elevated expression of PGC-1a, PPARa and PPARg. In parallel, metformin caused a marked reduction of serum TNF-a and CCL2 levels and of intrahepatic leukocytes, in particular T cells, NK cells and macrophages.
Conclusions: In a high-fat dietary model of NAFLD, Bcl-3 is a hepatocellular factor that promotes metabolic dysfunction and insulin resistance by suppressing transcription factors of hepatic lipo- and gluconeogenesis.

O067

HEMATOPOIETIC OVEREXPRESSION OF CYP27A1 REDUCES HEPATIC INFLAMMATION INDEPENDENTLY OF 27-HYDROXYCHOLESTEROL LEVELS IN LDLR$^{-/-}$ MICE VIA NPC-MODULATED CHOLESTEROL TRANSPORT

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Background and Aims: Non-alcoholic steatohepatitis (NASH) is characterized by hepatic lipid accumulation and inflammation. Currently, the underlying mechanisms leading to hepatic inflammation are still unknown. The breakdown of free cholesterol inside Kupffer cells (KCs) by the mitochondrial enzyme CYP27A1 produces 27-hydroxycholesterol (27HC). We recently demonstrated that administration of 27HC to hyperlipidemic mice reduced hepatic inflammation. In line, hematopoietic deletion of CYP27A1 resulted in increased hepatic inflammation. In the current manuscript, the effect of hematopoietic overexpression of CYP27A1 on the development of NASH and cholesterol trafficking was investigated. We hypothesized that CYP27A1 overexpression in KCs will lead to reduced hepatic inflammation.
Methods: Irradiated *Ldlr*$^{-/-}$ mice were transplanted (tp) with bone marrow from mice overexpressing CYP27A1 (*Cyp27a1*^{over}) and wild-type (Wt) mice and fed either chow or high-fat, high-cholesterol (HFC) diet for 3 months. Additionally, gene expression was assessed in bone marrow derived macrophages (BMDM) from *Cyp27a1*^{over} and Wt mice. Electron microscopy anaylis was performed to study intracellular cholesterol distribution.
Results: In line with our hypothesis, hepatic inflammation in HFC-fed *Cyp27a1*^{over}-tp mice was reduced and KCs were less foamy compared to Wt-tp mice. Remarkably, these changes occurred even though plasma and liver levels of 27HC did not differ between both groups. BMDM from *Cyp27a1*^{over} mice revealed reduced inflammatory gene expression and increased expression of cholesterol transporters compared to Wt BMDM after LPS stimulation. In addition, 27HC increased expression of NPC proteins, thereby reducing lysosomal cholesterol accumulation.
Conclusions: Our data suggest that overexpression of CYP27A1 in KCs reduces hepatic inflammation independently of 27HC levels in plasma and liver via increased NPC-mediated cholesterol transport. This study further points towards KCs as specific target for improving therapy of NASH.

O068

LYSOSOMAL CHOLESTEROL IN KUPFFER CELLS, PARTICULARLY WHEN OXIDIZED, CONTRIBUTES TO MURINE STEATOHEPATITIS

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Background and Aims: Recently, the importance of lysosomes within the metabolic syndrome, including fatty liver disease, is gaining increasing attention. It has been suggested that macrophages during atherosclerosis as well as Kupffer cells (KCs) during hepatic inflammation demonstrate properties of an acquired lysosomal storage disorder. So far, it is unclear whether there is a causal relationship between lysosomal cholesterol accumulation (LCA) in KCs and hepatic inflammation. Additionally, the specific contribution of the oxidized LDL (oxLDL) fraction to LCA, its concomitant effect on lysosomal function, and on hepatic inflammation is unexplored.
Methods: Hematopoietic deficiency of the mutant Niemann-Pick type C1 (NPC1^{mutant}) protein was used as a tool to induce LCA in KCs of hyperlipidemic mice. To induce high levels of anti-oxLDL antibodies, NPC1^{mutant}-transplanted mice were immunized every two weeks with heat-inactivated pneumococci until the end of the experiment.

Results: Compared to wildtype, NPC1^{mutant}-transplanted mice displayed severe hepatic inflammation and fibrosis. Anti-oxLDL immunization of NPC1^{mutant}-transplanted mice improved cholesterol metabolism, lysosomal dysfunction and liver inflammation, in contrast to non-immunized NPC1^{mutant}-transplanted mice.
Conclusions: A direct causal link exists between LCA in KCs and hepatic inflammation. Rather than total cholesterol, we specifically show that oxLDL significantly contributes to lysosomal dysfunction, cholesterol homeostasis and the hepatic inflammatory response.

O069
EVIDENCE FOR A ROLE OF CCR2 IN HUMAN NON-ALCOHOLIC FATTY LIVER DISEASE

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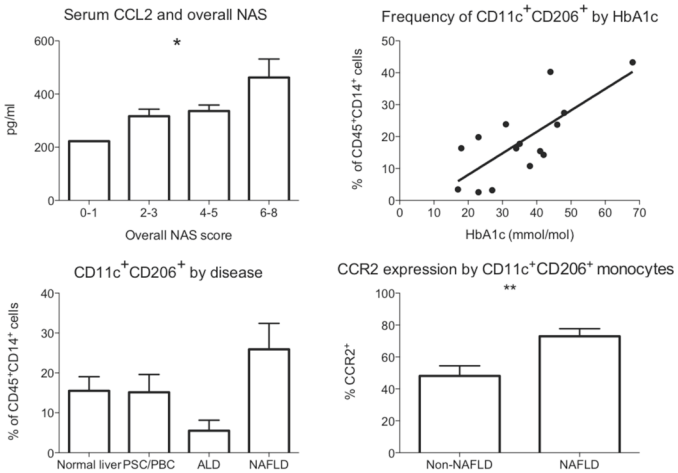
Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is closely associated with the metabolic syndrome. Chemokine-C-receptor 2 (CCR2) has been shown to be expressed on myeloid cells that infiltrate human adipose tissue in obese individuals where they play a role in causing insulin resistance. CCR2+ cells have been demonstrated in murine models of obesity and NAFLD. We sought to define the role of CCR2 in human fatty liver disease.
Methods: Serum and liver tissue was collected from patients at University Hospitals Birmingham NHS Foundation Trust with appropriate ethical approval, and from healthy volunteers. Serum concentration of CCL2 was assessed by ELISA and compared to histological assessment of liver disease. Whole liver tissue CCL2 gene expression was analysed by rt-PCR. CCR2 expression on liver-infiltrating cells was assessed with immunohistochemistry of paraffin embedded liver sections and flow cytometry of cells isolated from liver tissue.
Results: Serum CCL2 concentration was elevated in patients with NAFLD compared to healthy volunteers (mean concentration 330 ng/ml, SD 157.2 vs. 207 ng/ml SD 90.6, $p = 0.030$), where CCL2 concentration correlated with inflammation assessed by Kleiner-Brunt system (one way ANOVA $p = 0.025$). Gene expression of CCL2 was markedly upregulated in NASH cirrhosis compared to normal liver tissue (mean fold increase 56.5 $p < 0.01$). CCR2$^+$ cells were observed particularly around areas of steatosis in NAFLD liver tissue. Flow cytometry of liver infiltrating cells demonstrated CCR2 expression predominantly on CD14$^+$ monocytes. A subset of CD14$^+$ monocytes expressed CD11c and CD206, and was associated with insulin resistance (correlation with HbA1c r^2=0.56, $p < 0.001$). This subset was more frequent in NAFLD liver [% of CD14$^+$, 26.42% (SEM 4.7) vs. 14.31% (2.70), $p = 0.035$] where CCR2 expression was also greater [76.32% (3.32) vs. 45.48% (6.48), $p = 0.002$].



Conclusions: CCL2 expression is greater in NAFLD and correlates with inflammation. A subset of CCR2 monocytes associated with insulin resistance are seen more frequently in NAFLD. These results demonstrate a role for CCR2 in human fatty liver disease that complement observations made in murine models. Further work to investigate the effects of targeting this axis in humans may provide a novel therapeutic method for this common disease.

O070
CX3CR1 IS A GATEKEEPER FOR INTESTINAL BARRIER INTEGRITY: LIMITING STEATOHEPATITIS BY PROMOTING INTESTINAL HOMEOSTASIS

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) represents the most common liver disease in Western societies and is regarded as the hepatic manifestation of the metabolic syndrome. The G-Protein-coupled chemokine receptor CX3CR1 has been shown to play a central role in many metabolic syndrome related diseases including type-2 diabetes, atherosclerosis and obesity. However, the role of CX3CR1 in relation to NAFLD progression remains unclear. Recent data indicate that intestinal dysbiosis drives NASH development and CX3CR1 is essential for intestinal homeostasis. Based on these findings, we hypothesized that CX3CR1 plays a role in regulating the gut–liver axis and therefore has implications for the progression of NASH.
Methods: Male wildtype (WT) and CX3CR1$^{-/-}$ mice were fed either a normal chow diet (NCD), high-fat diet (HFD) or methionine-choline-deficient (MCD) diet to induce steatohepatitis. For eradication of the intestinal microbiota, the drinking water of the mice was supplemented with a broad-spectrum antibiotic cocktail.
Results: CX3CR1$^{-/-}$ mice demonstrated more pronounced liver steatosis, inflammation and glucose intolerance compared to controls. Surprisingly, the experimental diets induced a marked reduction of intestinal resident macrophages in CX3CR1$^{-/-}$ mice, which was associated with significant alterations in the intestinal microbiota composition, a leaky epithelial barrier and a reduced phagocytic capacity of intestinal macrophages. Collectively, these intestinal changes led to an increased translocation of endotoxin and stronger activation of pathogen recognition receptors (PRRs) in livers of CX3CR1$^{-/-}$ mice, thereby triggering an enhanced inflammatory response in the liver. Strikingly, depletion of the intestinal microbiota by administration of broad-spectrum antibiotics (AB) suppressed the number of infiltrating macrophages and promoted a restorative phenotype of liver macrophages. Consequently, AB-treated mice demonstrated a marked improvement of steatohepatitis and glucose tolerance.
Conclusions: CX3CR1 dampens the microbiota-mediated activation of the hepatic innate immune response and thus limits the progression of steatohepatitis. Hence, CX3CR1 is a gatekeeper by promoting intestinal homeostasis.
K.M. Schneider and V. Biegs contributed equally.

O071

TELOMERASE REVERSE TRANSCRIPTASE MUTATIONS ARE ASSOCIATED WITH HEPATOCELLULAR CARCINOMA IN NASH

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Background and Aims: In an increasing proportion of cases, hepatocellular carcinoma (HCC) develops in nonalcoholic steatohepatitis (NASH). Mutations in telomerase reverse-transcriptase (hTERT), the main regulator of telomere length, have been associated with a spectrum of progressive familial liver disease characterized by steatosis, and telomere attrition characterizes cirrhosis and cancer development. Aim was to determine whether shortened telomeres and functional hTERT mutations predispose to HCC in NASH.
Methods: In 40 consecutive Italian unrelated patients with HCC occurring in NASH-related liver disease (78% with cirrhosis), 40 patients with NASH-related cirrhosis, and 60 healthy subjects with normal liver enzymes, telomere length was evaluated by qRT-PCR and hTERT coding regions and intron-exon boundaries were analyzed by Sanger sequencing on DNA from peripheral blood leukocytes. Prediction of functional consequences of genetic variants was estimated in silico by consulting Telomere Database (http://telomerase.asu.edu) and bioinformatic algorithms.
Results: Telomere length was reduced in HCC patients (median: 1.01 IQR: 0.76–1.42; calculated as ratio of telomere repeat copy number to 36B4 single gene copy number) vs. cirrhosis (median: 1.12 IQR: 0.83–1.70;p = 0.1) and vs. healthy subjects (median: 1.40 IQR: 1.00–1.72;p = 0.002). We detected novel rare coding variants of hTERT in NASH HCC patients, while no mutations were found in those with cirrhosis and healthy controls (prevalence 10%; p = 0.040 vs. cirrhosis, p = 0.012 vs. healthy subjects; p = 0.001 vs. controls overall). Three HCC patients were carriers of missense mutations: Ala67Val in homozygosity and Pro193Leu in heterozygosity located in the N-terminal (template binding domain, possibly damaging), while one was heterozygous for the Glu668Asp (catalytic domain, likely damaging). One patient presented a heterozygous frameshift mutation (Glu113Arg_fs*79, likely damaging). All patients positive for hTERT mutations had cirrhosis and were carriers of the I148M PNPLA3 variant predisposing to HCC in NASH; mortality at 24 months was 100% (vs. 65% in negative patients).
Conclusions: These data suggest that multiple rare hTERT mutations may frequently contribute to the pathogenesis of HCC in NASH by favouring telomere attrition, with possible clinical implications for relatives of the probands. We are currently validating these findings in an independent European cohort.

Cirrhosis and complications 1

O072

STATIN USE SIGNIFICANTLY DECREASES DECOMPENSATION AND DEATH IN VETERANS WITH HEPATITIS C-RELATED COMPENSATED CIRRHOSIS

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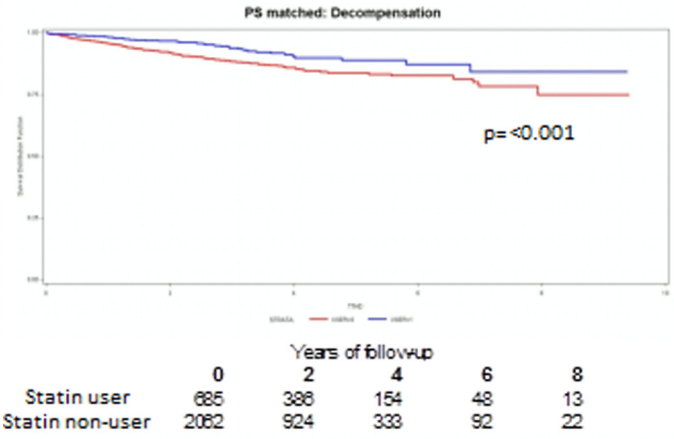
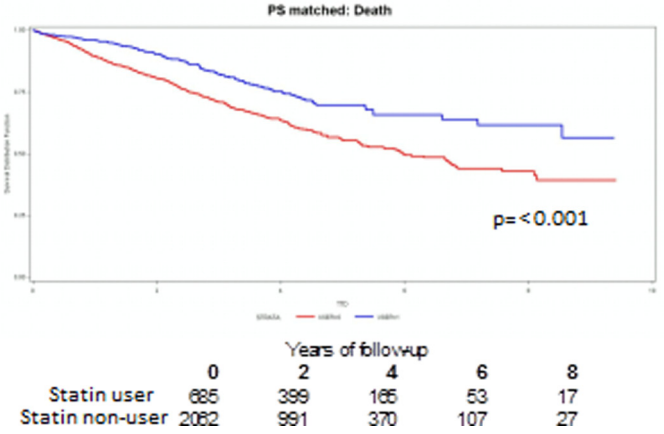
Background and Aims: Statin use has been shown to decrease portal pressure in patients with cirrhosis, and improve survival in patients who have bled from varices. However, the long-term effect of statin use on cirrhosis decompensation and mortality in patients with compensated cirrhosis is unknown.
Aim: To study the effect of statin use on decompensation and mortality in hepatitis C virus (HCV)-infected patients with compensated cirrhosis.
Methods: Using the Veteran Affairs clinical case registry from 01/1996 to 12/2009 (n = 342,157), we identified a cohort of HCV mono-infected patients with compensated cirrhosis and no statin use at baseline. New statin users (median time of use was 2.8 years) were propensity matched to up to 5 non-users. The propensity score was created using variables associated with statin prescription: demographics, calendar year of index visit, site of statin prescription, site prescribing pattern, number and type of lipid tests, laboratory data including liver tests, and





comorbid conditions. As previously validated, decompensation was defined by the presence of one inpatient or two outpatient ICD-9 codes for esophageal varices with bleeding, ascites and/or spontaneous bacterial peritonitis. The association between statin use and decompensation and death was estimated using cox proportional hazards model with adjustment for age + Child score and age + MELD score.

Results: Among 40,512 patients with HCV compensated cirrhosis (98% male, median age 56 years), 2,802 statin users were identified. From this group 685 statin users were propensity score matched with 2,062 non-users. Discrimination of propensity score model was excellent (c = 0.92). The figure shows plots of time to decompensation (A) or death (B) by statin use in the matched cohort. Statin use was associated with lower risk of decompensation [HR 0.55 (95% CI 0.39, 0.77)] or death [HR 0.56 (95% CI 0.46, 0.69)] compared to non-users. These findings persisted after adjustment for age + Child score [decompensation HR 0.53 (95% CI 0.37, 0.74); death HR 0.54 (95% CI 0.44, 0.66)] or age + MELD score [decompensation HR 0.53 (95% CI 0.38, 0.75); death HR 0.54 (95% CI 0.44, 0.66)].

Conclusions: Statin use in U.S. Veterans with HCV compensated cirrhosis is associated with over 40% lower risk of hepatic decompensation and death.

O073

NON-SELECTIVE BETA-BLOCKERS AND MORTALITY IN CIRRHOSIS PATIENTS WITH OR WITHOUT REFRACTORY ASCITES: POST HOC ANALYSIS OF THREE LARGE RCT'S WITH 1198 PATIENTS

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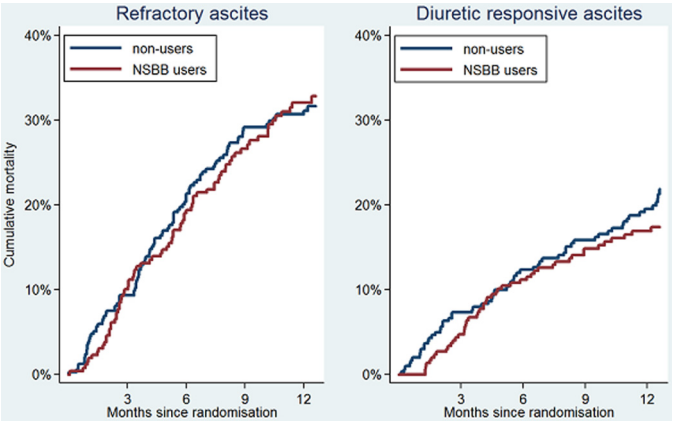
Background and Aims: Non-selective β-blockers (NSBB) are the standard treatment to prevent bleeding from esophageal varices, but their safety in patients with refractory ascites has been questioned. We used data from randomised trials to examine the effect of NSBB on mortality in cirrhosis patients with or without refractory ascites.

Methods: In 2006–2008, 1198 patients were enrolled in three multicentre randomised trials to determine the efficacy of satavaptan in treating ascites. Two of the trials were stopped early, but even so all participants' vital status was assessed after the planned 52 weeks of treatment. We used these data to compare the mortality of NSBB users vs. non-users. With Cox regression we adjusted for confounding by age, gender, MELD score, Child–Pugh score, serum sodium, history of variceal bleeding, cirrhosis aetiology and refractory ascites.

Results: NSBB users (n = 562) were more likely than non-users (n = 636) to have a history of variceal bleeding (30% vs. 13%), but they were less likely to have Child–Pugh class C cirrhosis (class A/B/C = 8%/68%/24% vs. 8%/64%/28%) or refractory ascites (46.4% vs. 52.5% among non-users of NSBB). MELD scores (median 12 [IQR 8–15] vs. 11 [IQR 8–15]) and serum sodium (median 137 [IQR 134–140] vs. 136 [IQR 133–139]) were similar in the two groups. During the follow-up (median follow-up for survival = 52.5 weeks) 129 NSBB users and 163 non-users died, and the cumulative mortality was 24.2% vs. 27.1%. Confounder adjustment did not change this overall impression that NSBB use did not affect mortality (adjusted HR = 0.92, 95% CI 0.72 to 1.18). When we examined patients with refractory ascites separately, NSBB use still had no effect on mortality (n = 595, adjusted HR = 1.02, 95% CI 0.74 to 1.39). Nor did it affect mortality among patients with diuretic-responsive ascites (n = 603, adjusted HR = 0.78, 95% CI 0.53 to 1.16) (Figure). We also examined the effect of NSBB in subgroups defined by a daily NSBB dose ≥80 mg or <80 mg, a MELD score ≥18 or <18, a mean arterial pressure ≥82 mmHg or <82 mmHg, or with or without a history of spontaneous bacterial peritonitis: NSBB use did not increase mortality in any of these subgroups.

Conclusions: NSBB use did not affect mortality in these cirrhosis patients with ascites.



O074

A METABOLOMIC STUDY OF SERUM FROM PATIENTS WITH CIRRHOSIS IDENTIFIES 2 METABOLITES THAT ACCURATELY PREDICT THE ACUTE HVPG RESPONSE TO β-BLOCKER THERAPY

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Background and Aims: Complications of cirrhosis (ascites, encephalopathy, variceal bleeding) are related to the presence of portal hypertension (PHT). A decrease in hepatic venous pressure gradient (HVPG) greater than 10% (HVPG responders) after the acute i.v. administration of propranolol is associated with a lower risk for these complications to appear and with a better survival. However, only about a half of patients are HVPG responders to propranolol and, up to now, there are not non-invasive markers to identify them. Thus, an invasive study of HVPG is needed. The present study aims at identifying a metabolomic serum profile in patients with cirrhosis and PHT that allows predicting a good HVPG response to acute i.v. propranolol.

Methods: 40 patients with cirrhosis (Child–Pugh A/B/C=21/12/7) and HVPG≥10 mmHg, in whom HVPG response to i.v. propranolol was assessed, were prospectively included. HVPG was measured at baseline and 20 minutes after i.v. propranolol (0.15 mg/kg). A targeted metabolomic analysis of serum samples using UPLC-MS (ultra-performance liquid chromatography coupled to mass spectrometry; 562 metabolites) was performed. The best combination of metabolites to identify HVPG responders (HVPG reduction >10%) was obtained using stepwise logistic regression (LR) from the subset of metabolites with a p value <0.1. Baseline clinical variables associated to a good response to propranolol were also studied.

Results: 25 of 40 patients (63%) were HVPG responders. The metabolomic study identified 65 metabolites that were significantly different among HVPG responders and non-responders. LR analysis identified 2 lipidic metabolites that showed a good prediction of the response to propranolol (AUC=0.872, CI=0.754–0.989), being a robust model at internal cross-validation (mean AUC 0.841). This model had a specificity of 80%, sensibility of 84%, Positive PV of 88% and Negative PV of 75% to identify HVPG responders, with a

positive and negative likelihood ratio of 4.2 and 0.2, respectively. The addition of clinical variables to the model did not improve its performance.

Conclusions: the combination of 2 lipidic serum metabolites accurately identifies patients with a good response to the acute administration of propranolol. The analysis of these metabolites could be a useful non-invasive tool to identify these patients.

O075

ASSESSMENT OF THE TRANSJUGULAR PORTO-SYSTEMIC SHUNT (TIPSS) IN THE MANAGEMENT OF COMPLICATIONS OF NONCIRRHOTIC PORTAL HYPERTENSION

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Background and Aims: Noncirrhotic portal hypertension (NCPHT) is a heterogeneous group of diseases characterized by portal hypertension without cirrhosis or a cause of chronic liver disease and no venous obstruction. The efficacy of the TIPSS in this population has not been reported.

Methods: We analyzed the charts of patients with NCPHT undergoing a TIPSS procedure in 5 reference centers between 1994 and 2013. Survival, presented as means ± SD, was assessed using the log-rank test and the Cox model.

Results: Thirty-eight patients (16 F, 22 M, aged 45±16 years) were included. Associated conditions were prothrombotic states in 9 patients (protein C and S deficiency, myeloproliferative disease, pregnancy, antiphospholipid syndrome), immune disorders in 12 (inflammatory myositis, rheumatoid arthritis, HIV, common variable immune deficiency, Crohn's disease), exposure to toxins in 9 (oxaliplatine, hematopoietic stem cell graft) and idiopathic in 8. Patients were grouped as low-risk thrombophilia or idiopathic (n = 12) and others (n = 26). Indication for TIPSS was uncontrolled variceal hemorrhage in 22 patients, refractory ascites in 12, exsudative enteropathy in 1, hepatic hydrothorax in 1 and preparation for abdominal surgery in 2. The porto-systemic gradient decreased from 20±7 to 6±3 mmHg. During follow-up (average 34±32 months), 10 patients died 16±20 months after TIPSS insertion, 5 from complications of the procedure or of the liver disease and 5 from the associated conditions. In the refractory ascites group, 6/12 (50%) died, including 5 in the first 6 months. Among patients with uncontrolled hemorrhage, 7 (31%) rebled, including 3 with early TIPSS thrombosis. Fourteen patients (38%) were hospitalized for encephalopathy. Using univariate analysis, serum creatinine ($p = 0.003$), MELD score ($p = 0.006$) and ascites as the indication ($p = 0.03$) at the time of the procedure predicted death. Survival was higher in the group of patients with low-risk thrombophilia or idiopathic NCPHT than in the other group ($p = 0.04$). Among deceased patients, mean MELD score was 14±5 and Child–Pugh score ranged from 6 to 9 at the time of the procedure.

Conclusions: Outcome was good after TIPSS in NCPHT patients with low-risk thrombophilia or idiopathic disease. Patients with bleeding as the indication for TIPSS had good long-term outcome despite frequent rebleeding. In patients with refractory ascites, TIPSS is associated with a high short-term mortality.

O076

CARDIAC VOLUME OVERLOAD AND PULMONARY HYPERTENSION AFTER LONG-TERM FOLLOW-UP IN TIPS PATIENTS

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Background and Aims: Insertion of transjugular intrahepatic portosystemic shunt (TIPS) might induce significant cardiac overload and in rare cases lead to acute cardiac decompensation. Yet little is known about long-term cardiac outcome. Therefor we conducted a study to analyze cardiac function in long-term follow-up after TIPS.

Methods: Between 2000 and May 2014, patients with liver cirrhosis who underwent successful TIPS creation, were seen for follow-up or underwent TIPS creation in another hospital while waiting for liver transplantation at the University Hospital Heidelberg were included. Findings on echocardiography from the last one before and first one at 1 to 5 years after TIPS were analyzed and included. A control group of patients with liver cirrhosis were matched to TIPS patients with available echocardiography after TIPS. Results are expressed as median and interquartile range. For comparison Mann–Whitney and Wilcoxon's test were used.

Results: The final study cohort consists of 158 (103 male) patients after 23 were excluded due to BCS and 51 because no follow-up after TIPS was available. Median age at TIPS was 56 years (49–64) and 29 patients were Child A, 92 Child B and 37 Child C at time of TIPS.

Significant change was found for pulmonary-arterial systolic pressure (PASP) increasing from 25 mmHg (22–33) to 30 mmHg (25–36) ($p = 0.038$; $N = 22$) and septal thickness ($p = 0.009$; $N = 34$) as well as for left atrial diameter (LAD) ($p = 0.001$; $N = 33$) changing from 37 mm (33–43) to 40 mm (36–47) and end-diastolic diameter (EDD) ($p < 0.001$; $N = 33$). No differences were found for the end-systolic diameter ($p = 0.266$; $N = 23$), posterior wall ($p = 0.354$; $N = 32$), early to late diastolic tissue velocity ratio (E'/A') ($p = 0.123$; $N = 9$) and right ventricular diameter ($p = 0.562$; $N = 12$).

Comparison of echocardiographic findings between TIPS ($N = 55$) and control group ($N = 110$) revealed a significantly larger LAD ($p < 0.001$), VCI ($p = 0.009$), EDD ($p = 0.004$) and an increased PASP ($p < 0.001$) in the TIPS group. Incidence of pulmonary hypertension (PH) in the TIPS group was 6 out of 158 (3.8%) patients during mean follow-up of 28.6 (±27.8) months whereas in the control group no patient developed PH during mean follow-up of 24.3 (±22.8) months.

Conclusions: In conclusion, TIPS is accompanied by long-term effects on cardiac function in cirrhotic patients. Signs of volume overload after insertion of TIPS is seen even after >1 year, causing pulmonary hypertension in some patients. Therefor we recommend regular echocardiography in the long-term follow-up in these patients.

O077

USE OF DIRECT ORAL ANTICOAGULANTS (DOACS) IN PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS AND/OR CIRRHOSIS

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Background and Aims: DOACs are increasingly used off label in patients with splanchnic vein thrombosis and/or cirrhosis. Since efficacy and safety remain a concern in these patients, our aim was to describe the indications of DOACs, the reasons for switching from other anticoagulants and the possible adverse effects.
Methods: A questionnaire including demographic information, laboratory data, DOACs treatment characteristics and complications was sent to the members of the VALDIG Consortium from 42 centers in 15 countries.
Results: Thirty-three centers (79%) answered the questionnaire. The use of DOACs in patients with splanchnic vein thrombosis (SVT) and/or cirrhosis was reported for 35 cases (8 centers in 6 European countries). Patients (21 men/14 women) were aged 52 (range 16–82 years). Indications for anticoagulation were SVT (22), peripheral deep vein thrombosis (1), Budd-Chiari syndrome (3), atrial fibrillation (5) and others (4). The DOACs used were rivaroxaban (31), dabigatran (3) and apixaban (1) for a median of 7 months (range 1–42). The reasons for choosing DOACs were no need for INR monitoring (17), to avoid injections (4), previous complications of other anticoagulants (7) and unknown (7). There were 13 patients with cirrhosis and median Child–Pugh score B7 (range 6–9), MELD 8 (range 7–13). In cirrhotic patients the median daily dose of rivaroxaban was 15 mg (n = 11), of apixaban 10 mg (n = 1) and of dabigatran 220 mg (n = 1).
During treatment, creatinin, bilirubin, albumin, platelet counts and ALT did not change significantly, while INR increased from 1.72 to 2.19 (p = 0.002).
Adverse events included 1 case of anemia probably due to portal hypertensive gastropathy, 1 case of dizziness and disorientation and 1 case of progression of SVT. In 1 patient rivaroxaban was stopped due to hepato-renal syndrome.
Conclusions: A limited number of patients with SVT and/or cirrhosis are currently treated with DOACs. The most frequent reason for choosing DOACs is no need for INR monitoring. DOACs seem to be safe because no major bleeding episodes or liver deterioration occurred during treatment, but there is a compelling need for more data in this field.

O078

THE PREVALENCE AND MORTALITY OF ACUTE-ON-CHRONIC LIVER FAILURE DEFINED BY APASL VS. EASL–CLIF CONSORTIUM: A MULTICENTER, RETROSPECTIVE COHORT STUDY IN KOREA (KACLIF STUDY)

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Background and Aims: Acute-on-chronic liver failure (ACLF) is defined differently between Eastern (APASL) and Western countries (EASL-CLIF consortium). This study aimed to investigate the prevalence of ACLF according to the APASL vs. EASL-CLIF definitions as well as short-term mortality.
Methods: We collected data for 1470 hospitalized patients (male 1092, median age 55±12 years) with chronic liver disease (CLD) and acute decompensation (AD) from January 2013 to December 2013 from 21 academic hospitals in Korea.
Results: The most common underlying cause of CLD was alcohol (63.1%) and the main forms of decompensation were ascites (33.0%) and variceal bleeding (32.2%). The prevalence of ACLF development based on the APASL and EASL-CLIF consortium were 155 (10.5%) and 197 (13.4%) at admission, and 45 (3.1%) and 77 (5.2%) within 28 days of enrollment, respectively. The 28-day and 90-day mortality were higher in patients with ACLF at enrollment than in those without ACLF at enrollment (by APASL definition: 19.4% vs. 4.3%, and 35.7% vs. 6.6%, respectively, $P < 0.001$; by EASL-CLIF definition: 32.0% vs. 3.9%, and 46.5% vs. 8.4%, respectively, $P < 0.001$). Of the 207 patients who satisfied the APASL or EASL-CLIF definition at the time of admission, only 54 (26.1%) patients satisfied both definitions, while the remaining patients (73.9%) satisfied only one (with APASL definition, 92 patients; with EASL-CLIF definition, 61 patients). In patients with ACLF, the 28-day and 90-day mortality in patients who satisfied both definitions were significantly higher than in those who satisfied just one definition (44.4% and 53.7%, respectively). The enrolled patients with previous AD within 1 year had higher short-term mortality than those with no previous AD or AD more than 1 year before (28-day mortality: 10.1% vs. 7.8% vs. 5.8%, $P = 0.037$, 90-day mortality: 20.2% vs. 12.0% vs. 8.8%, $P < 0.001$). And, of the 118 AD patients at enrollment who had chronic liver disease but not cirrhosis, the 28-day mortality in patients with ACLF at enrollment was not statistically different from that in patients without ACLF at enrollment (by APASL definition: 11.1% vs. 2.8%, $P = 0.275$, by EASL-CLIF definition: 6.3% vs. 2.9%, $P = 0.446$).
Conclusions: Development of ACLF is associated with high short-term mortality. However, the prevalence and mortality are significantly different when ACLF is defined by the two different APASL vs. EASL-CLIF definitions. Thus, refinement of the two ACLF definitions or a consensus definition is urgently needed.

O079

CALR SOMATIC MUTATIONS IN A PROSPECTIVE COHORT OF 308 PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS

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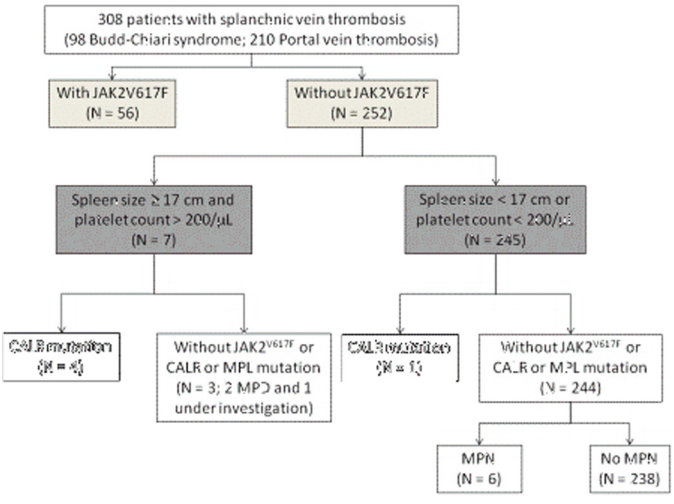
Background and Aims: Myeloproliferative neoplasms (MPN) are the leading cause of splanchnic vein thrombosis (SVT). Due to its excellent specificity for MPN, JAK2V617F analysis is very helpful in this setting. Still, 5 to 10% of SVT patients have a JAK2V617F-negative MPN, requiring bone marrow biopsy and/or cultures of erythroid progenitors for diagnosis. Recently, somatic mutations of the calreticulin (*CALR*) gene have been described in MPN patients. The aim of this study was to determine the prevalence as well as the features associated with CALR mutations in a large prospective cohort of SVT patients.
Methods: CALR, JAK2V617F and MPL mutations were analyzed in all patients with SVT prospectively included in our center. Bone marrow biopsy (searching for clusters of dystrophic megakaryocytes) and/or cultures of erythroid progenitors (searching for endogenous colonies) were performed when spleen size was ≥17 cm by imaging and platelet count >200/μL (Chait, Brit J Haematol 2005) or when considered relevant by the physician.
Results: 308 patients (162 F, 146 M), aged 43±16 years have been included (Figure). Facteur II or V gene mutations were detected in 18 (6%) and 20 (7%) patients, respectively. Antiphospholipid syndrome and paroxysmal nocturnal haemoglobinuria were diagnosed in 31 (10%) and 5 patients (2%), respectively. A MPN was diagnosed in 69 patients (23%), including 56 with JAK2V617F. None of the 308 patients had MPL mutation. CALR mutations were found in 5 (2%) patients (3F, 2M; aged 33±5 years; 4 idiopathic myelofibrosis and 1 essential thrombocythemia). All 5 patients had a spleen size ≥17 cm, except for one who had a spleen size of 16.6 cm. None of the 5 patients had JAK2V617F, factor II or V gene mutation, protein C, S or antithrombin deficiency, antiphospholipid syndrome or paroxysmal nocturnal haemoglobinuria. Patients with CALR mutations had more commonly a portal vein involvement than other patients (5/5 *vs.* 145/300; p = 0.028).



Conclusions: CALR mutations are detected in 2% of SVT patients. When present, these mutations are the only risk factor for thrombosis suggesting that this is a major determinant for SVT. CALR mutations should thus be investigated in patients with SVT without JAK2V617F. These results reinforce the diagnostic strategy based on spleen size and platelet counts since a MPN is present in virtually all JAK2V617F-negative patients with a spleen size ≥17 cm and platelet count >200/μL, *vs.* only in 3% of other patients.

Autoimmune and genetic liver disease

O080

EARLY CLINICAL FEATURES ASSOCIATED WITH LONG-TERM RISK OF TRANSPLANTATION IN PRIMARY SCLEROSING CHOLANGITIS: RESULTS FROM THE UK-PSC CONSORTIUM

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Background and Aims: Early assessment of new therapies in PSC will be aided by better surrogates of disease outcome. We determined baseline and follow up factors associated with transplantation in a representative UK cohort.
Methods: Detailed phenotypic data were collated on patients recruited to the UK-PSC national cohort (August 2008-July 2014). Unadjusted hazard ratios (HR) were estimated using the proportional hazards model; adjusted HRs were estimated using forward selection to identify variables associated with transplantation (Stata 11.2/SE).
Results: From a 1700 patient cohort, detailed phenotypic data were analysed in 500 patients (77 hospitals; 5 transplant centres; 59.4% male). Median age at diagnosis was 49.5 years, median follow-up 5.7 yrs. 68.4% had IBD (75.1% UC), 13.4% had another autoimmune disease. 87% were prescribed ursodeoxycholic acid (UDCA) (mean dose 12.8 mg/kg/day). 100 (20%) had a transplant (median age 51 yrs); need for transplantation was associated with cholangiographic disease burden with only 9.4% of patients without cholangiographic changes at baseline undergoing transplantation. 14% of females had transplants compared to 23.9% of males. 9.2% died without transplant, 4.4% due to PSC. 8.8% developed a GI cancer, most commonly colorectal (4.2%) and cholangiocarcinoma (3%). By univariate analysis, factors associated with increased need for transplantation were baseline ALP >2×ULN (p = 0.005; HR = 1.87, 95% CI 1.21, 2.89) and male gender (p = 0.03; HR 1.63, 95% CI 1.05, 2.53). At 1 and 2 years post diagnosis, ALP >1.5 and >2×ULN were associated with transplantation need (p < 0.001; see table). Absence of cholangiographic changes at baseline was protective (p = 0.023; HR 0.38, 95% CI 0.16, 0.87). After multivariate analysis, ALP >2×ULN at baseline (p = 0.001; HR 2.32, 95% CI 1.43, 3.77) and year 2 (p = 0.015; HR 2.96, 95% CI 1.23, 7.13) and ALP >1.5×ULN at year 1 (p < 0.001; HR 2.92, 95% CI 1.85, 4.59) remained associated with need for transplantation. Absence of cholangiographic changes

(p = 0.008; HR 0.28, 95% CI 0.11, 0.72) and older age at diagnosis (p = 0.01; HR 0.98, 95% CI 0.97, 1.0) appeared protective. Of note, UDCA use and IBD had no effect on transplantation need.

Variable	Univariate		Multivariate	
	p	HR (95% CI)	p	HR (95% CI)
Age at diagnosis	0.088	0.99 (0.98, 1.00)	0.01	0.98 (0.97, 1.00)
Gender	0.03	1.63 (1.05, 2.53)		
IBD	0.537	1.15 (0.73, 1.82)		
Autoimmune disease	0.90	0.97 (0.61, 1.77)		
UDCA use	0.94	1.03 (0.55, 1.92)		
Intra- and extra-hepatic disease		1		
Intra-hepatic disease only	0.006	0.52 (0.33, 0.83)	0.027	0.57 (0.35, 0.94)
No cholangiographic changes	0.023	0.38 (0.16, 0.87)	0.008	0.28 (0.11, 0.72)
Baseline ALP >1.5×ULN	0.155	1.39 (0.88, 2.19)		
Baseline ALP >2.0×ULN	0.005	1.87 (1.21, 2.89)	0.001	2.32 (1.43, 3.77)
Year 1 ALP >1.5×ULN	<0.001	2.57 (1.67, 3.95)	<0.001	2.92 (1.85, 4.59)
Year 1 ALP >2.0×ULN	<0.001	2.49 (1.63, 3.79)		
Year 2 ALP >1.5×ULN	<0.001	2.71 (1.58, 4.66)	0.059	1.96 (0.97, 3.96)
Year 2 ALP >2.0×ULN	<0.001	2.53 (1.55, 4.11)	0.015	2.96 (1.23, 7.13)

Conclusions: Long-term outcome in patients with PSC is associated with cholangiographic disease burden and ALP at baseline and follow up, with no effect of UDCA use. These emerging data from a representative UK cohort help inform models for disease stratification and choice of early exploratory endpoints for clinical trials.

O081
VAP-1 IS ELEVATED IN PSC, CORRELATES WITH CLINICAL OUTCOME AND EXHIBITS AMINE OXIDASE ACTIVITY IN A SUBSTRATE-DEPENDENT MANNER

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Background and Aims: Vascular adhesion protein (VAP)-1 is an adhesion molecule possessing potent amine-oxidase activity. Activation on hepatic sinusoidal endothelium (HSEC) leads to H_2O_2 production, NFκB activation and expression of the gut-homing receptor mucosal addressin cell-adhesion molecule-1, which promotes homing of gut-tropic lymphocytes to the liver. Given the proposed role this pathway has in hepatic disorders complicating inflammatory bowel disease (IBD), our aim was to quantify circulating soluble (sVAP-1) titre in blood and determine intrahepatic/colonic enzyme activity in patients with primary sclerosing cholangitis (PSC)/IBD, and investigate consequences of activating VAP-1 with variant amine substrates.
Methods: Soluble VAP-1 was measured by ELISA in blood samples from patients with PSC (n = 105); primary biliary cirrhosis/PBC (n = 60); autoimmune hepatitis/AIH (n = 99); IBD-alone (n = 50) and healthy controls (n = 21). Correlations with clinical outcome were assessed using Cox proportional hazards assumption and KM estimates. Protein lysates were extracted from (a) explanted liver (PSC=9, PBC=10, AIH=5, normal donor, n = 10); and (b) colonic resections (ulcerative colitis=7) and VAP-1 activity quantified using the Amplex-Red assay. Putative VAP-1 substrates were selected based on their inclusion in the human metabolome database (www.hmdb.ca).
Results: PSC patients had higher circulating VAP-1 concentrations (median 517 ng/mL) than those with AIH (475), PBC (472), IBD-alone (413) and healthy controls (425) (p < 0.0001). VAP-1 levels >540 ng/mL in PSC were associated with significantly worse transplant-free survival (HR:2.94, p = 0.008). Intrahepatic VAP-1 enzyme activity was significantly greater in patients with PSC (227pmol H_2O_2/min/mg protein) compared with PBC (124), AIH (128) and healthy controls (109) (p < 0.001) and comparable to activity in inflamed colonic tissue (220). The substrate associated with highest VAP-1 enzymatic efficiency was cysteamine (k_{cat}^{app}/K_m^{app}: 5.4×10^7); an amine secreted by Escherichia spp. which induces colitis in the murine colon.
Conclusions: High levels of circulating sVAP-1 in PSC are predictive of poor prognosis. Intrahepatic VAP-1 enzyme activity is also increased in PSC akin to that seen in inflamed colon. The ability of VAP-1 to catabolise amine substrates secreted by gut commensals/enteric pathogens, provides a theoretical link between altered colonic microbiota and mucosal immunity in the pathogenesis of PSC.

O082
THE GUT MICROBIOTA IN PRIMARY SCLEROSING CHOLANGITIS DIFFERS FROM THAT OF HEALTHY CONTROLS AND ULCERATIVE COLITIS PATIENTS WITHOUT BILIARY DISEASE

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Background and Aims: The gut microbiota has been hypothesised to influence primary sclerosing cholangitis (PSC) because of its role in bile acid homeostasis and the strong association between PSC and inflammatory bowel disease (IBD). We aimed to characterize the gut microbiota of PSC in a cross-sectional cohort.
Methods: After excluding participants with antibiotics the last 4 weeks, elimination diets and previous bowel resections, we extracted DNA from stool samples collected from 90 PSC patients (n = 58 [64%] with concurrent IBD), 263 healthy controls (HC) and 36 ulcerative colitis (UC) patients without PSC. The median age (male %) for PSC patients was 50 (63), UC patients 40 (44) and HC 46 (41). All groups had similar body mass index. The V3-V4 region of the 16S rRNA gene was then sequenced from all stool samples on the Illumina MiSeq platform. Quantitative Insights Into Microbial Ecology (QIIME) was used for subsequent quality control and analysis. PSC patients and HC were randomized to a discovery and a replication panel for a two-stage design, followed by a joint analysis for comparison with UC patients.
Results: The PSC patients and HC exhibited large global differences in microbiota composition as measured by beta diversity in both the discovery and replication panel (unweighted UniFrac, PERMANOVA: p = 0.001). In PSC, there was reduced alpha diversity (i.e. richness and evenness) compared to HC in both panels (phylogenetic diversity, Chao1 and Shannon index, p < 0.001). On the taxonomic level, 12 bacterial genera had significantly different abundance in PSC and HC in the discovery panel (p < 0.05), out of which 6 were validated in the replication panel. In the joint analysis PSC patients differed from both HC and UC patients in regard to beta diversity (p < 0.01), whereas both PSC and UC differed from HC in alpha diversity (p < 0.001), with no differences between UC and PSC. In PSC patients, however, the IBD status did not influence beta and

alpha diversity. Most genera separating PSC and HC were similar in PSC and UC. However, PSC patients exhibited a significantly increased abundance (2.8 fold) of the *Veillonella* genus compared to both HC and UC ($p < 0.02$).
Conclusions: The fecal microbial profile in PSC patients was different from HC and UC patients without PSC, while the profiles in PSC patients with and without IBD were similar. Compared with HC and UC, PSC patients exhibited a marked increase in abundance of the *Veillonella* genus, which has also been linked to other conditions of fibrosis.

O083
ABSENCE OF BSEP/ABCB11 PROTECTS FROM CHOLESTATIC LIVER AND BILE DUCT INJURY IN A MOUSE MODEL OF SCLEROSING CHOLANGITIS

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Background and Aims: Cholestasis, characterized by intrahepatic accumulation of potentially cytotoxic bile acids (BAs) leads to liver injury reflected by disruption of hepatocellular integrity, inflammation and fibrosis. Bile salt export pump (BSEP/ABCB11) is the main canalicular BA transporter and therefore rate limiting step for hepatobiliary BA secretion. Here we investigate the role of ABCB11 in development of cholestatic liver and bile duct injury in a model of sclerosing cholangitis.
Methods: Wildtype (WT) and ABCB11 knockout (KO) mice were subjected to common bile duct ligation (CBDL) (7 days) and DDC feeding (4 weeks) as models for acute and chronic cholestasis (sclerosing cholangitis), respectively. RNA profiling was performed by RT-PCR. BA transporter expression was assessed at protein level. Serum biochemistry, hepatic hydroxyproline (HP) levels, BA composition as well as liver histology were assessed.
Results: In contrast to WT, ABCB11 KO mice where protected from cholestatic liver injury after CBDL or DDC feeding, reflected by unchanged serum levels of ALT, AST, AP, total cholesterol and BA. ABCB11 KO mice where protected from inflammation (reflected by unchanged mRNA levels of F4/80 and MCP1) and fibrosis (reflected by unchanged mRNA levels of Col1a1, Col1a2 and aSMA protein levels) while WT mice display significant up-regulation of both inflammatory (F4/80 4-fold and Mcp1 60-fold) and fibrotic (Col1a1 2-fold, Col1a2 2.5-fold, HP levels 2-fold) markers. Polyhydroxylated BAs (PHBA) were increased 4-fold in ABCB11 KO CBDL mice compared to WT CBDL mice ($p < 0.01$). mRNA expression of Cyp2b10, downstream target of CAR, a nuclear receptor regulating BA detoxification, was increased (10-fold) in ABCB11 KO mice under cholestatic conditions. Protein levels of BA transporter such as NTCP, OATP (sinusoidal uptake) and MRP2 (canalicular export) were reduced in WT and increased in ABCB11 KO mice under cholestatic conditions. MRP3 and MRP4 (sinusoidal export) protein levels were increased in WT CBDL mice and not changed in ABCB11 KO mice. Notably, the phosphorylated form of STAT3 – essential for the differentiation of pro-inflammatory TH17 cells – was exclusively found in WT BDL mice.
Conclusions: Changes in BA metabolism favouring detoxification and efflux of potentially toxic BAs protects ABCB11 KO mice from development of acute and chronic cholestatic liver injury. Therefore PHBAs may open a new therapeutic avenue against cholestasis and subsequent progression toward fibrosis in cholangiopathies such as PBC and PSC.

O084
NOVEL TREATMENT OPTIONS TO IMPROVE ABERRANT PRE-MESSENGER RNA SPLICING AND PROTEIN FOLDING IN ATP8B1 DEFICIENCY

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Background and Aims: ATP8B1 deficiency is a severe autosomal recessive liver disease due to mutations in the *ATP8B1* gene and characterized by intrahepatic cholestasis. Many *ATP8B1* mutations are predicted to affect pre-messenger RNA splicing. The most common missense mutation (p.I661T) leads to protein misfolding and disturbed protein homeostasis (proteostasis). Current therapeutic options are insufficient. The aim of our study was to elucidate the molecular consequences of *ATP8B1* mutations at exon-intron boundaries and the development of mutation-specific therapies for ATP8B1 deficiency.
Methods: Fourteen *ATP8B1* mutations at exon-intron boundaries, associated with ATP8B1 deficiency, were analysed for their effect on pre-messenger RNA splicing using an *in vitro* minigene system. Modified versions of U1 small nuclear RNA (snRNA) matching donor splice sites were expressed and subsequent splice rescue was evaluated by reverse transcription PCR. The potential of 13 proteostasis regulators to restore ATP8B1 p.I661T plasma membrane expression was evaluated by cell surface biotinylation.
Results: Eleven mutations resulted in aberrant splicing and a complete absence of correctly spliced product. Three mutations led to partially incorrect splicing. Expression of modified U1 snRNA complementary to the mutated splice donor sites strongly improved or completely rescued splicing for several *ATP8B1* mutations located at donor, as well as acceptor, splice sites. Furthermore, six proteostasis regulators caused a significant upregulation of ATP8B1 p.I661T plasma membrane expression.
Conclusions: The majority of *ATP8B1* mutations at exon-intron boundaries resulted in total exon skipping and compensatory modified U1 snRNAs were able to improve exon definition very efficiently. In addition, proteostasis regulators were able to improve ATP8B1 p.I661T plasma membrane expression. Modified U1 snRNAs as well as proteostasis regulators could be a novel therapeutic strategy in ATP8B1 deficiency and other genetic diseases.

O085
LANREOTIDE REDUCES LIVER VOLUME YET ACCELERATES MUSCLE WASTING AND WEIGHT LOSS IN SYMPTOMATIC POLYCYSTIC LIVER DISEASE

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Background and Aims: Polycystic liver disease (PCLD) can induce malnutrition due to extensive hepatomegaly, forming an indication for liver transplantation (LTx). The somatostatin analogue (SA) lanreotide 120 mg (LAN) given for 6 months reduces liver volume (LV). We aimed to investigate the efficacy and long-term safety of LAN.

Methods: In this 18 month prospective trial performed in 2 tertiary centers, patients were administered LAN 90 mg/4 weeks for 6 months. If a LV reduction ≥100 mL (responders) was induced, patients continued to receive LAN 90 mg for an additional year. Non-responders were offered dose-escalation to LAN120 mg. LV and body composition were measured by CT-scan.
Results: Of 67 patients screened, 59 were included. 21/53 patients (40%) were responders at month 6 of which 19 (90%) reached the 18-month endpoint. The absolute LV change after 18 months was −430 mL (SE: 92). In non-responders, LV increased with +120 mL (SE: 42), yet no further increase was observed after dose-escalation in the 24 patients who reached the endpoint. All subscales of the PCLD complaints specific assessment (POLCA) improved including an improved food intake ($p = 0.04$). A significant decrease of weight and total muscle mass was observed; whereas subcutaneous and visceral fat mass were not affected. This was congruent with a significant reduction of insulin-like growth factor 1 level: from 19% to 50% below the age-adjusted normal range level ($p = 0.002$).
Conclusions: This study demonstrates that low doses of LAN induce LV reduction and relief of complaints. Weight loss and muscle wasting may limit the long-term use of SA due to the induction of growth-hormone deficiency and needs further exploration (clinicaltrials.gov.identifier NCT01315795).

O086
BUDESONIDE FOR AUTOIMMUNE HEPATITIS: RESPONSE RATE AND LIMITATIONS IN A LARGE REAL LIFE COHORT

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Background and Aims: Autoimmune hepatitis (AIH) responds well to immunosuppressive treatment with prednisolone and azathioprine, yet many patients (pts) suffer from steroid-specific side effects. Budesonide was shown to be effective in inducing and maintaining remission with fewer steroid-specific side effects in a controlled clinical trial (Manns et al. 2010 Gastroenterology). In order to assess the usefulness of budesonide in real life we here report on a large cohort of pts with AIH or AIH/PBC overlap syndrome treated with budesonide at our unit.
Methods: 83 pts (67 AIH and 16 overlap) were identified who had received budesonide for at least 6 months (mos). Treatment response was evaluated at 6, 12 and 24 mos. For comparison with the published study, treatment response was defined as normal levels of aminotransferases.
Results: Start of treatment was with 9 mg/d and the mean maintenance dose of budesonide was 6.2 mg. 38 (46%) pts were given budesonide due to steroid-specific side effects, 35 (42%) because of steroid-dependency and 10 (12%) pts as initial therapy. After 6, 12 and 24 mos, 55%, 69% and 64% of all pts achieved remission. Pts who were given budesonide due to steroid-specific side effects, demonstrated remission in 58% after 6 mos, in 64% after 12 mos and in 64% after 24 mos. The steroid-dependent group reached remission in 54% after 6 mos, in 70% after 12 mos and in 63% after 24 mos. The group of pts who were given budesonide as initial therapy achieved remission in 50% after 6 mos, in 85% after 12 mos and in 67% after 24 mos. At last follow up, 66% (55/83) of all pts were in remission. A total of 36 pts (43%) were still on budesonide, but 22 pts (26%) had changed back to prednisolone. 13 pts received neither prednisolone nor budesonide and 9 pts had stopped treatment. We identified 13 pts with steroid-induced osteopenia at the beginning of budesonide treatment. In follow up DXA we saw improvement in 5 pts, stable results in 5 pts and worsening in only 1 pt. 18 pts were treated with budesonide alone, including 8 pts with an overlap. Overlap pts responded favourably to budesonide treatment with 75% reaching remission after 6 mos compared to only 40% of AIH pts.
Conclusions: Budesonide can be considered as an alternative to conventional steroids for the treatment of AIH. However, efficacy may be lower than prednisolone since one quarter of pts had to be switched back due to insufficient response. Pts with an overlap syndrome may demonstrate a more favourable response to budesonide treatment.

O087
IgG4+ B-CELL RECEPTOR CLONES IN PERIPHERAL BLOOD DISTINGUISH IgG4-ASSOCIATED CHOLANGITIS/AUTOIMMUNE PANCREATITIS FROM PRIMARY SCLEROSING CHOLANGITIS

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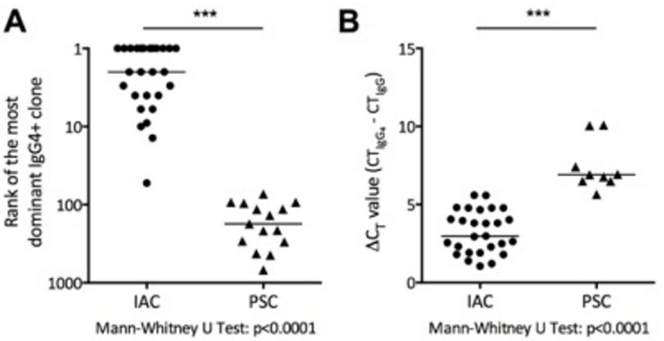
Background and Aims: Immunoglobulin G4-related disease (IgG4-RD) predominantly affects the biliary tract (IgG4-associated cholangitis, IAC) and pancreas (autoimmune pancreatitis, AIP). Its clinical presentation often mimics primary sclerosing cholangitis (PSC) and/or pancreatobiliary malignancies, and an accurate diagnostic marker is lacking. Recently, we identified dominant IgG4+ B-cell receptor (BCR) clones in blood of 6 patients with active IAC, but not in controls, using next-generation sequencing (NGS; Hepatology 2013;57:2340). Here, we aimed to validate our findings in larger patient cohorts and to test a novel qPCR protocol in comparison to NGS.
Methods: 27 patients with IAC/AIP fulfilling HISORt criteria were included in one Dutch (n = 17) and one English (n = 10) academic hospital. Also, 15 PSC controls were included. The total BCR heavy-chain repertoire was analysed using primers for all V(ariable)-genes and total RNA from peripheral blood. All amplified products encoded CDR3, the region defining a unique clone. The number of identical sequences reflects the size of the BCR clone. In addition, qPCR was performed on 27 IAC/AIP patients and 9 disease controls using forward primers for all IgG subtypes and IgG4 specifically; one generic reverse primer was used within the constant region of the receptor. Differences between the specific IgG4 and total IgG message in peripheral blood were calculated and expressed as differences in CT value.



Results: IgG4+ BCR clones were detected in all individuals included. 25/27 of all IAC/AIP patients had IgG4+ BCR clones that were among the 10 most dominant IgG+ BCR clones in blood (median 2nd, range 1–53; Figure 1A), whereas the most dominant IgG4+ BCR clone in

PSC patients ranked 176th (median, range 73–686, $p < 0.0001$). All IgG4+ clones together covered 16.6% of all IgG+ clones, and 19.9% of the IgG+ repertoire (compared to 1.6% and 1.0% resp. in PSC controls). This difference was confirmed using qPCR: in IAC/AIP patients the median ΔCT value was 3.0 (1.0–5.6), compared to 6.9 in PSC patients (range 5.6–10.1, $p < 0.0001$).

Conclusions: Identification of highly dominant IgG4+ BCR clones by NGS in patients with active IAC/AIP clearly distinguishes IAC/AIP from PSC. Our novel qPCR protocol, in comparison to NGS, could be a simple and inexpensive alternative diagnostic tool. Expansion of patient cohorts (IAC/AIP, PSC, pancreatobiliary malignancies) for test validation is underway.

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G13

OMBITASVIR/PARITAPREVIR/RITONAVIR FOR TREATMENT OF HCV GENOTYPE 1b IN JAPANESE PATIENTS WITH OR WITHOUT CIRRHOSIS: RESULTS FROM GIFT-I

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Background and Aims: Sustained virologic response (SVR) rates of 99–100% have been achieved with the 3 direct acting antivirals ombitasvir (OBV), paritaprevir (PTV, formerly ABT-450, identified by AbbVie and Enanta, co-dosed with ritonavir [PTV/r]) with dasabuvir (DSV) in multinational phase 3 clinical trials of treatment-naïve (TN) and treatment-experienced (TE) HCV genotype 1b (GT1b)-infected patients (pts). Since PTV levels are higher in Japanese than Western pts, the Phase 3 trial, GIFT-1, evaluated the efficacy and safety of PTV/r with OBV (2D) without DSV in Japanese HCV GT1b-infected pts.

Methods: In the double-blind (DB), placebo (PBO)-controlled substudy, TN and TE adult Japanese HCV GT1b-infected pts without cirrhosis were randomized 2:1 to receive DB co-formulated 2D (OBV/PTV/r; 25 mg/150 mg/100 mg QD) or PBO for 12 weeks. Pts with cirrhosis received 12 weeks of open-label (OL) 2D. SVR was defined as HCV RNA < lower limit of quantification (<25 IU/ml) 12 weeks post-treatment (SVR12). The primary efficacy population (PEP) was TN, non-cirrhotic pts eligible for interferon (IFN) therapy with HCV RNA ≥100,000 IU/ml. Treatment-emergent adverse events (TEAEs) were captured from first dose through 30 days after last dose of study drug.

Results: A total of 363 pts were randomized/enrolled (DB 2D, N = 215; DB PBO, N = 106; OL 2D, N = 42). Demographics and baseline disease characteristics were generally similar between DB treatment arms. Among non-cirrhotic pts, SVR12 rates were 94.6% in the PEP, 94.2% in TN, and 96.1% in TE pts. SVR12 rates were >90% in all subpopulations based on stratification factors at randomization (Tables). Reasons for not achieving SVR12 are shown in the table. Most TEAEs were mild or moderate in severity. The most common TEAEs in non-cirrhotic pts were nasopharyngitis (16.7%), headache (8.8%), and peripheral edema (5.1%). Peripheral edema occurred significantly more frequently with DB 2D (5.1%) than PBO (0%) and all pts were using concomitant calcium-channel blockers. Nasopharyngitis (14.3%) was the most common TEAE in cirrhotic patients. Of the patients receiving DB or OL 2D, 9 had serious TEAEs and 3 discontinued treatment due to TEAEs.

Table 1. Virologic response in HCV GT1b-infected patients treated with ombitasvir/paritaprevir/r

Population (ITT)	SVR12 [#], n/N (%)	95% CI
Non-cirrhotic treatment-naïve patients	131/139 (94.2)	89.1, 97.1
Primary efficacy population	106/112 (94.6)	90.5, 98.8
Low viral load [¶]	6/6 (100)	61.0, 100
IFN ineligible [¶]	21/23 (91.3)	73.2, 97.6
Non-cirrhotic treatment-experienced patients	73/76 (96.1)	89.0, 98.6
Relapse	21/22 (95.5)	78.2, 99.2
Non-response	28/28 (100)	87.9, 100
IFN intolerant	24/26 (92.3)	75.9, 97.9
Cirrhotic patients	38/42 (90.5)	77.9, 96.2

IFN, interferon.
[#] Patients with missing data after imputations were applied were counted as failures.
[¶] These categories are not mutually exclusive.

Table 2. Reasons for not achieving SVR12

	Non-cirrhotic ITT population		Cirrhotic ITT population
	Treatment naïve (N = 139)	Treatment experienced (N = 76)	All patients (N = 42)
On-treatment virologic failure*, n	0	1	1
Relapse [†], n	4	1	2
Other [‡], n	4	1	1

*Confirmed viral breakthrough or failure to suppress with at least 6 weeks of treatment.
[†] Confirmed HCV RNA ≥25 IU/mL post-treatment through SVR12 out of the patients completing treatment with HCV RNA <25 at end of treatment and post treatment HCV RNA data.
[‡] Patients not achieving SVR12 without on-treatment viral failure or relapse (e.g. early discontinuation or lost to follow-up).

Conclusions: High response rates were achieved with the IFN- and RBV-free once-daily regimen of OBV/PTV/r in Japanese HCV GT1b-infected pts with or without cirrhosis. The 2D regimen was generally well-tolerated with few treatment discontinuations due to TEAEs.

G14

SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) IS A MAJOR DETERMINANT OF TREATMENT RESPONSE TO TERLIPRESSIN FOR HEPATORENAL SYNDROME TYPE 1 (HRS-1)

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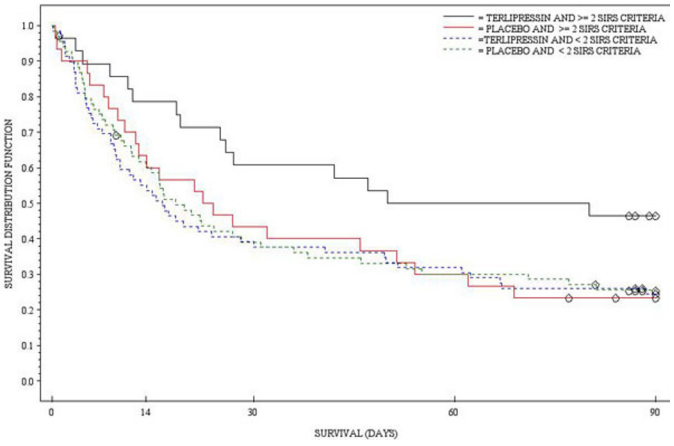
Background and Aims: Infection is a common precursor of HRS-1 and may be associated with SIRS. The presence of SIRS in patients with decompensated cirrhosis and acute kidney injury (AKI) has been associated with a poorer prognosis, and therefore possibly also a lower response to treatment for AKI. The REVERSE study, a randomized placebo controlled study of terlipressin in patients with HRS-1 included enrollment of patients with ≥2 SIRS criteria in the

absence of untreated infection. The aim of the current study was to evaluate the effect of SIRS on the response to terlipressin in HRS-1.
Methods: SIRS was defined as the presence of ≥2 of 3 SIRS criteria: WBC <4000 or >12,000 cells/μL; HR >90 bpm and HCO_3 <21 mmol/L (an approximation of the SIRS criterion PaCO2 of <32 mmHg). Subjects with HRS-1 were randomized to terlipressin (1 mg IV every 6 hours) or placebo, plus albumin in both groups. Efficacy endpoints evaluated included confirmed HRS reversal (CHRSR) defined as 2 SCr values ≤1.5 mg/dL, at least 48 hours apart, and HRS reversal defined as a decrease in SCr to ≤1.5 mg/dL, change in SCr and survival.
Results: SIRS was present in 58/196 (30.0%) of subjects. Baseline clinical characteristics were similar between SIRS and non-SIRS groups; there was slightly higher WBC and heart rate, and slightly lower HCO3 in the SIRS group, as expected. In the SIRS group, CHRSR was observed in 9/28 subjects (32.1%) with terlipressin vs. 1/30 subjects (3.3%) with placebo ($p < 0.005$). In subjects treated with terlipressin (n = 97), CHRSR was observed in 9/28 subjects with SIRS (32.1%) vs. 10/69 subjects (14.5%) without SIRS ($p < 0.05$); HRS reversal with terlipressin was observed in 12/28 (42.9%) subjects with SIRS compared with 11/69 (15.9%) subjects without SIRS ($p < 0.005$). The change from baseline to end of treatment in SCr in response to terlipressin was significantly greater for SIRS subjects (−1.4 mg/dL) vs. non-SIRS subjects (−0.8 mg/dL, $p < 0.05$). For subjects treated with terlipressin, survival estimates (Figure) and the percentage of subjects alive and transplant-free at Day 90 were higher in the SIRS subjects (13/28, 46.4%) compared to the non-SIRS subjects (17/69, 24.6%) ($p = 0.0517$)
Conclusions: SIRS is common in patients with HRS-1. Despite the presence of SIRS, the response of these subjects to terlipressin is significantly better than that in subjects without SIRS and those treated with placebo. Terlipressin is effective in improving renal function and achieving HRS reversal in patients with HRS-1 and SIRS.



G15

THE ASSOCIATION OF SOFOSBUVIR AND DACLATASVIR FOR TREATING SEVERE RECURRENCE OF HCV INFECTION AFTER LIVER TRANSPLANTATION: RESULTS FROM A LARGE FRENCH PROSPECTIVE MULTICENTRIC ANRS CO23 CUPILT COHORT

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Background and Aims: HCV recurrence is a main complication following liver transplantation (LT) impacting graft and patient survival. The recent approval of IFN-free regimen using direct antiviral agents (DAA) has radically changed the management of liver transplant recipients. However, the optimal strategy remains to be determined. The aim of this study was to assess efficacy and safety of sofosbuvir (SOF) and daclatasvir (DCV)-based regimens in this setting.
Methods: The ANRS CO23 CUPILT study is a prospective cohort including currently 335 patients (pts) with HCV recurrence and treated with 2nd generation DAA. All pts treated with SOF+DCV±ribavirin (RBV) between Jul. 2013 and Nov. 2014 were included in the present study.
Results: This study enrolled 190 liver recipients (male: 76%, mean age 59±9years [33–83]), with an active HCV recurrence (G1:116, G3:17, G4:12, G5:1), in 19 centers. The mean delay between LT and starting therapy was 74.2±73.5 months [1.8–339.4]. Treatment duration was 24 weeks (wk) in 84% of pts. RBV was given in 67 (38%) pts. Indication was severe HCV recurrence, including 32 cirrhotic pts, and 11 cholestatic hepatitis. Eighty-three (46%) pts were non-responders to a previous course of therapy post-LT [including Peg-FN/RBV: 64 (77%); 1st generation PI based regimen: 16 (19%)]. Eighty-nine percent of pts received cyclosporine (32%) or tacrolimus (68%).
At baseline, HCV viral load, GGT and hemoglobin levels were 6.4±0.7log10 IU/mL [4.2–8.4], 381.8±573.4 IU/L [16–3561], 12.8±2.3 g/dL [7.8–17.9], respectively. After 4 wks, a complete virological response was obtained in 36/178 (23%) pts. One pt experienced a virological breakthrough (wk 8); among 115 pts who have completed the treatment, the end of treatment response was achieved in 114 (99%). One pt relapsed at wk 4 post-treatment; 62/64 (97%) and 42/44 (95%) pts achieved SVR4 and 12, respectively.
Twenty-three (12%) pts experienced serious adverse events (SAE), leading to 2 premature discontinuations (wk 1 and 2) including 1 death (diabetic coma). Most common SAE were hematological toxicity. Anemia occurred in 6 (3%) pts, all treated with RBV. Eight non-severe infections occurred. Two non-severe biopsy-proven acute rejections occurred after treatment discontinuation. No drug–drug interaction was reported.

Conclusions: SOF and DCV based regimens showed excellent results combining high rate of SVR4 and 12 (97% and 95%, respectively) and a good tolerance. Final results will be presented.

G16

THE COUNFOUNDING ROLE OF SEVERE COMORBIDITIES AND ALCOHOL USE DISORDERS ON PROGNOSIS IN CHRONIC HEPATITIS C VIRUS INFECTION: AN ANALYSIS OF THE 2008–2012 FRENCH NATIONAL HOSPITAL DISCHARGE DATABASE

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Background and Aims: There is an epidemiological relationship between chronic hepatitis C virus (HCV) infection and alcohol use disorders (AUD). AUD is a leading cause of liver disease and death. However, burden of chronic HCV infection analyses barely take into account the potential confounding role of AUD on prognosis. Our aim was to compare the prognostic value of chronic HCV infection and AUD in the general population.
Methods: In 2008–2012, 28,953,755 adult individuals residing in Metropolitan France were hospitalized and 1,506,453 died at hospital (51.6% and 55.7% of National Vital Statistics, respectively). We characterized all hospitalized patients by severe comorbidities (see Table), and tracked their trajectory according to chronic HCV infection and/or AUD (including withdrawal/abstinence). Age at death was analyzed in multivariate Cox proportional hazards model from January 2008 to last discharge or transplantation with stratification by gender, main French regions, and having received care in teaching hospitals.
Results: Chronic HCV infection was present in 112,146 (0.39%) of hospitalized patients, AUD in 705,259 (2.44%), both chronic HCV infection and AUD in 23,351 (0.08%; i.e., 20.8% of HCV and 3.3% of AUD). Overall, the adjusted hazard ratio of in-hospital death (aHR) was 1.90 (95% confidence interval 1.86–1.94) for chronic HCV infection and 3.13 (3.10–3.15) for AUD, with a negative interaction effect between chronic HCV infection and AUD (aHR: 0.93; 0.90–0.97). Alcohol withdrawal or abstinence was significantly associated with lower mortality risks (HR, 0.66; 0.65–0.67). Subgroup analyses by severe comorbidities revealed that chronic HCV infection was only associated with higher mortality risks in presence of severe comorbidities (Table: groups 1 to 4, and 7 to 12). In absence of severe comorbidities, the prognostic value of chronic HCV infection was not statistically significant among patients with cirrhosis and milder liver disease stage (groups 13 to 17). In contrast, AUD was associated with higher mortality risks in all prognostic subgroups, including all liver disease stages.
Conclusions: AUD has a dismal prognostic value in the general population and in the minority group of patients with chronic HCV infection. Alcohol withdrawal and abstinence increase survival regardless of HCV treatment.

Table (abstract G16): Sequential characterization of patients

	Characteristic	Patients, n (%)	HCV (%)	AUD (%)	HCV & AUD (%)	In-hospital death (%)	Mortality risk, aHR (95% CI) HCV	AUD	Interaction HCV & AUD	Withdrawal or abstinence among AUD
1.	HIV/AIDS	91,931 (0.32)	15.1	6.2	2.8	5.3	**2.21 (2.05–2.38)**	**2.55 (2.27–2.85)**	0.99 (0.85–1.15)	**0.53 (0.43–0.65)**
2.	Liver transplant before Jan. 2008	7,552 (0.03)	18.4	21.5	4.1	15.3	**1.28 (1.07–1.53)**	**2.17 (1.87–2.52)**	0.92 (0.68–1.24)	1.03 (0.75–1.40)
3.	Kidney transplant before Jan. 2008	28,615 (0.10)	4.1	1.9	0.2	9.6	**2.09 (1.80–2.43)**	**2.21 (1.77–2.76)**	0.91 (0.53–1.56)	0.85 (0.47–1.52)
4.	Other solid organ transplant before Jan. 2008	6,367 (0.02)	1.6	4.1	0.3	17.9	**1.63 (1.03–2.55)**	**1.42 (1.05–1.91)**	0.21 (0.03–1.58)	0.62 (0.26–1.47)
5.	Allogeneic stem cell transplant before Jan. 2008	3,530 (0.01)	1.3	2.0	0.1	25.9	0.83 (0.43–1.63)	0.98 (0.59–1.62)	2.64 (0.62–11.25)	1.24 (0.40–3.80)
6.	Liver cancer	86,097 (0.30)	9.8	35.5	3.9	55.6	0.96 (0.92–0.99)	**1.25 (1.23–1.28)**	**1.07 (1.00–1.14)**	**0.73 (0.69–0.77)**
7.	Non-Hodgkin lymphoma	104,027 (0.36)	0.9	2.4	0.1	26.0	**1.29 (1.14–1.46)**	**1.62 (1.50–1.74)**	0.87 (0.59–1.29)	**0.74 (0.60–0.91)**
8.	Other cancer	2,361,576 (8.16)	0.4	4.0	0.1	26.9	**1.20 (1.15–1.25)**	**1.55 (1.53–1.57)**	1.00 (0.92–1.10)	**0.81 (0.79–0.83)**
9.	Cryoglobulinemia	3,460 (0.01)	26.2	9.5	5.1	10.5	**1.64 (1.26–2.15)**	**2.59 (1.66–4.05)**	0.75 (0.39–1.44)	0.76 (0.28–2.03)
10.	Chronic kidney disease	648,726 (2.24)	0.7	4.5	0.1	24.2	**1.52 (1.43–1.62)**	**2.10 (2.05–2.15)**	**1.21 (1.06–1.39)**	**0.70 (0.66–0.75)**
11.	Other severe comorbidity (Charlson Index)	4,057,570 (14.01)	0.5	4.8	0.1	11.9	**1.46 (1.40–1.53)**	**2.32 (2.29–2.36)**	**1.32 (1.21–1.44)**	**0.65 (0.62–0.67)**
12.	End-stage liver disease	122,215 (0.42)	3.2	36.4	1.9	18.6	**1.72 (1.54–1.92)**	**3.38 (3.27–3.49)**	**0.60 (0.53–0.69)**	**0.46 (0.43–0.49)**
13.	Cirrhosis	45,658 (0.16)	11.9	56.2	3.9	5.2	*0.97 (0.75–1.25)*	**4.44 (3.95–4.99)**	**0.58 (0.40–0.84)**	**0.32 (0.26–0.41)**
14.	Liver fibrosis	54,330 (0.19)	3.1	3.9	0.4	0.6	*1.40 (0.68–2.87)*	**2.41 (1.43–4.09)**	2.11 (0.61–7.29)	1.28 (0.50–3.30)
15.	Metabolic syndrome	1,756,391 (6.07)	0.3	2.3	0.0	0.9	*1.06 (0.77–1.46)*	**3.52 (3.23–3.83)**	0.58 (0.23–1.48)	**0.72 (0.58–0.89)**
16.	Injecting drug use	60,774 (0.21)	6.8	30.2	3.1	0.7	*0.90 (0.55–1.47)*	0.93 (0.73–1.19)	**2.13 (1.14–4.00)**	**0.59 (0.40–0.87)**
17.	None of the above	19,514,936 (67.40)	0.2	1.1	0.0	0.5	*0.98 (0.84–1.14)*	**3.94 (3.79–4.09)**	1.24 (0.86–1.78)	**0.58 (0.52–0.64)**

Notes: HCV, hepatitis C virus; AUD, Alcohol use disorders; aHR, adjusted hazard ratio.

ORAL PRESENTATIONS

G17

A DISTINCT PROFILE OF LYSO-PHOSPHATIDYLCHOLINES AND AMINO ACIDS CHARACTERIZES NAFLD IN LEAN SUBJECTS

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is typically associated with obesity and the metabolic syndrome, however, approximately 10% of lean subjects (BMI <25) also have NAFLD. The aim of this study was to identify clinical and metabolic features of NAFLD in lean Caucasian subjects.
Methods: Data from 247 patients allocated to one of 4 groups according to BMI and hepatic steatosis on ultrasound were obtained: lean healthy (BMI ≤25 kg/m^2, no steatosis, N = 76), lean steatosis (BMI ≤25 kg/m^2, steatosis, N = 57), obese healthy (BMI ≥30 kg/m^2, no steatosis, N = 52), obese steatosis (BMI ≥30 kg/m^2, steatosis; N = 62). A detailed clinical and laboratory examination including oral glucose tolerance test (oGTT) was performed. Metabolite profile was obtained by API 4000 triple quadrupole mass spectrometer (ABSciex) using the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences). Significant differences between groups were calculated using the false discovery rate (FDR) approach for metablomics analyses and ANOVA for comparison of clinical characteristics.
Results: Lean NAFLD subjects had fasting glucose concentrations and HOMA-IR similar to lean healthy subjects. However, lean NAFLD subjects had markedly impaired glucose tolerance as assessed by oGTT similar to obese NAFLD patients, and significantly different from lean healthy subjects (P < 0.001). In the metabolomics analysis significantly lower levels of sphingomyelin (OH) C14:1, lysophosphatidylcholine (lysoPC) C18:0, lysoPC C17:0, phosphatidylcholine with diacryl residue sum (PC aa) C34:2 and higher levels of glutamic acid (FDR < 0.001 for all analytes) were found in lean NAFLD compared to lean healthy subjects. A sum of lysoPC C18:0 and lysoPC C17:0 can separate lean healthy from lean NAFLD with a ROC area under the curve (AUC) of 0.76. Additionally, in lean NAFLD subjects higher levels of phosphatidylcholine with acyl-alkyl residue sum (PC ae) C 42:3, lysine and lower levels of alanine, tyrosine, valine and butyrylcarnitine (FDR < 0.001 for all analytes) were found when compared to obese NAFLD. ROC analysis of lysine, alanine and tyrosine discriminated lean and obese NAFLD subjects with an AUC of 0.88.
Conclusions: Although lean NAFLD patients have normal fasting glucose concentrations, the degree of glucose intolerance is similar to obese NAFLD patients. Furthermore, a distinct profile of lysoPCs and amino acids may be distinguishing indicators of the metabolic alterations linked to NAFLD in lean subjects.

G18

THE FXR AGONIST PX20606 REDUCES LIVER DAMAGE, FIBROSIS AND PORTAL HYPERTENSION IN CIRRHOTIC RATS

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Background and Aims: Steroidal FXR agonists attenuate liver injury and reduce portal hypertension in experimental models of cirrhosis. We aimed to assess the impact of the nonsteroidal FXR agonist PX20606 (PX) on liver histology, inflammation and hemodynamics in cirrhotic rats.
Methods: Cirrhosis was induced by carbontetrachloride (CCl_4) injections (2× weekly i.p. for 12 weeks) in Sprague Dawley rats. Controls received corn oil. PX (10 mg/kg/day) or placebo (DMSO) were gavaged daily from week 4. Blood was sampled at week 6 and 11. At end of treatment, mean arterial pressure (MAP), heart rate (HR), portal pressure (PP) and superior mesenteric artery blood flow (SMABF) were measured. Liver fibrosis was assessed by Sirius Red area (SRA) and content of hydroxyproline (HP), collagen-I (Col1) and α-smooth muscle actin (αSMA). Col1 and αSMA were assessed by immunofluorescence staining. Hepatic expression of fibrogenic genes was quantified by qRT-PCR [shown as x-fold (×) of control].
Results: CCl_4 rats presented with marked cirrhosis, elevated transaminases and portal hypertension compared to corn oil-treated animals. In CCl_4 rats, PX treatment significantly ameliorated fibrosis (SRA: 6.99±3.15 vs. 3.97±1.64%; p < 0.001. HP: 415±86 vs. 134±14 μg/g liver; p = 0.002) and reduced Col1 (12.28±1.78 vs. 4.93±0.28; p < 0.05) and αSMA (7.78±1.41 vs. 2.08±0.49; p < 0.05) content. Accordingly AST (555±30 vs. 227±83 IU/ml; p < 0.001) and ALT (538±233 vs. 193±86 IU/ml; p = 0.008) significantly decreased under PX treatment, while plasma cholesterol or triglycerides levels remained unaffected. In cirrhotic rats, PX treatment significantly decreased PP (11.9±1.4 vs. 9.7±1.4 mmHg; p = 0.037) and increased SMABF (8.88±2.62 vs. 13.81±2.81 ml/min/100g; p = 0.021), while not affecting MAP (125±14 vs. 116±25 mmHg) nor HR (330±29 vs. 333±52bpm). Livers of CCl_4-PX rats significantly overexpressed FXR target genes including bile salt export pump (2.5×), small heterodimer partner (2.3×), cystathionase (2.1×) and dimethylargininase (1.7×). Expression of endothelin-1 (0.45×), PDGF-Rβ (0.51×) and αSMA (0.61×) were significantly reduced. In corn oil-treated animals PX did not affect liver histology, transaminases, MAP or HR.
Conclusions: PX20606 treatment reduces hepatic inflammation, stellate cell activation and fibrogenesis and thus significantly reduced portal pressure in cirrhotic rats. PX20606, as a selective,

Table 1 (abstract G18). Results overview

	Corn-DMSO vs. Corn-PX20606			CCl_4-DMSO vs. CCl_4-PX20606		
	Corn Oil – DMSO	Corn Oil – PX20606	p-value	CCl_4 – DMSO	CCl_4 – PX20606	p-value
Sirius Red area [%]	0.61±0.3	0.56±0.3	**<0.001**	6.99±3.15	3.97±1.64	**<0.001**
Hydroxyproline content [μg/g liver]	161±11	175±3	**0.023**	415±86	134±14	**0.002**
collagen-I [%]				12.28±1.78	4.93±0.28	**<0.05**
α-SMA [%]				7.78±1.41	2.08±0.49	**<0.05**
AST [IU/ml]	114±26	108±13	**<0.001**	555±30	227±83	**<0.001**
ALT [IU/ml]	57±6	63±10	**0.014**	538±233	193±86	**0.008**
Cholesterol [mg/dL]	66±17	80±21	0.890	64±8	76 ±10	0.068
Triglycerides [mg/dl]	188±61	87±14	**0.044**	110±29	91±65	0.569
MAP [mmHg]	109±26	120±13	0.287	125±14	116±25	0.409
Heart rate [bpm]	314±45	299±37	0.517	330±29	333±52	0.867
SMABF [ml/min]	40.8±13.2	67.2±9.9	0.175	45.5±4.7	68.7±13.9	**0.008**
PP [mmHg]	6.9±0.7	8.8±1.3	**<0.001**	11.9±1.4	9.7±1.4	**0.037**

nonsteroidal FXR agonist represents a novel therapeutic option against liver fibrosis and portal hypertension.

Cirrhosis and complications 2

O088

HIGH-THROUGHPUT SEQUENCING OF THE HUMAN HEPATIC PROGENITOR CELL NICHE REVEALS DIFFERENT SIGNALLING PATHWAYS DEPENDING ON THE UNDERLYING CHRONIC LIVER DISEASE

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Background and Aims: Hepatic progenitor cells (HPCs) can differentiate into cholangiocytes or hepatocytes, depending on the underlying aetiology. Though some pathways are known to be involved in the process of differentiation, it still is unclear how HPCs interact with their surrounding niche.
Methods: Using laser microdissection, the HPC niche was isolated from samples obtained from patients diagnosed with primary sclerosing cholangitis (PSC n = 6) or hepatitis C virus (HCV n = 5), as models for biliary or hepatocellular regeneration. Isolated mRNA was amplified using the Clonetech SMARTer kit and processed for Illumina HiSeq sequencing. Differentially expressed genes were integrated with Ingenuity Pathway analysis. Immunohistochemistry was used for protein validation on a set 40 FFPE samples (PSC n = 20, HCV n = 20).
Results: In total 304 genes were significantly differentially expressed. Recruitment and homing of inflammatory cells was distinctly different. The niche in PSC was characterised by neutrophil attractant chemokines (CXCL5, CXCL6, IL-8) together with CCL28, whereas HCV was identified by T- and B-lymphocyte infiltration and a strong interaction with macrophages (as HPCs in HCV expressed the macrophage receptor MARCO). In addition the composition of the niche's extracellular matrix differed depending on the disease (e.g. FN1, LAMC2 in PSC and COL17A1 in HCV) but also reflecting the stage of the disease. Even the neighbouring endothelial cells proved to have a different phenotype (e.g. RORB, FOXJ1, INHBA). Transcriptional regulators (e.g. KLF4, ERK1/2, HNF4A) and growth factors (e.g. IGF2, TGFB2, BMP9) were also differentially expressed.
Conclusions: Our data sheds light on the different signalling pathways in human biliary vs hepatitis chronic liver disease.

O089

INCREASED NEURONAL EXPRESSION OF K-TYPE GLUTAMINASE POTENTIATES BRAIN EDEMA IN ACUTE LIVER FAILURE MICE THROUGH A TLR4 DEPENDENT PATHWAY

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Background and Aims: *Introduction:* In the brain Ammonia is detoxified to glutamine through the action of astrocytic glutamine synthase. This glutamine transported to the neurons serves as an energy source via neuronal mitochondrial glutaminase (K-glutaminase) producing ammonia. Accumulating ammonia may become neurotoxic, an effect partly modulated by TLR4. Therefore increasedbrain K-glutaminase activity and ammonia generation could produce neuronal dysfunction.
Aim: To assess the expression and activity of neuronal K-glutaminase in ALF and determine whether this involves TLR4-dependent pathways.
Methods: *Study 1*: 3 groups of mice studied. Healthy: n = 6; ALF induced by acetaminophen (APAP) (500 mg/kg IP): n = 7; and ALF treated with STM 28, a TLR4 antagonist. The animals were sacrificed after 8-hour or at coma. *Study 2*: 4 groups of mice studied [*WT control*: n = 10, *WT* (NH_3), n = 10 (using NH_4Cl in diet); *TLR4 ko control* ($TLR4^{-/-}$), n = 6; $TLR4^{-/-}$(NH_3) n = 13]. Arterial ammonia and frontal cortex brain water were measured. Brain was snap frozen for PCR and brain sections were stained for Glase.
Results: *Study 1*: ALF induced significant increase in ammonia and brain water in the APAP group compared with controls (345±32 vs. 132±11 umol/L, p = 0.002) (83.6±2.3% vs. 76.3±2.6%, p = 0.05) respectively. Immunohistochemistry of brain slices showed significantly increased K-Glase expression in the neurons in the frontal cortex, hippocampus and cerebellum of the ALF treated animals compared with healthy controls. Glase activity was significantly increased in ALF treated groups compared with controls (7.9±0.2 vs 6.2±0.7 p < 0.03) and reduced significantly in the STM 28 animals (p < 0.03) in parallel with reduced brain water and time to coma (p < 0.01 each). *Study 2*: Hyperammonemia resulted in a significant increase in brain water in WT animals compared with $TLR4^{-/-}$ cohort (p 0.02), Glase expression and activity was significantly reduced in the $TLR4^{-/-}$ hyperammonemic animals compared with the WT hyperammonemic group (8.5±0.4 vs 7.2±0.1, p = 0.01).
Conclusions: The study shows for the first time a potential role of neuronal production of ammonia by increased expression of Glase. Reduction of brain inflammation using TLR4 antagonist or a knocking out TLR4 results in reduction of Glase expression, activity and reduction in brain edema suggesting a potential role of TLR4 signalling in the modulation of increased neuronal Glase in ALF. Further studies should address the mechanisms involved and whether Glase may be a target of therapy.

O090

HUMANIZATION OF GERM-FREE MICE WITH ALCOHOLIC CIRRHOTIC MICROBIOTA, BUT NOT HEALTHY MICROBIOTA, INDUCES BACTERIAL TRANSLOCATION AND A PRO-INFLAMMATORY MILIEU, WHICH IS AMELIORATED WITH LACTOBACILLUS GG

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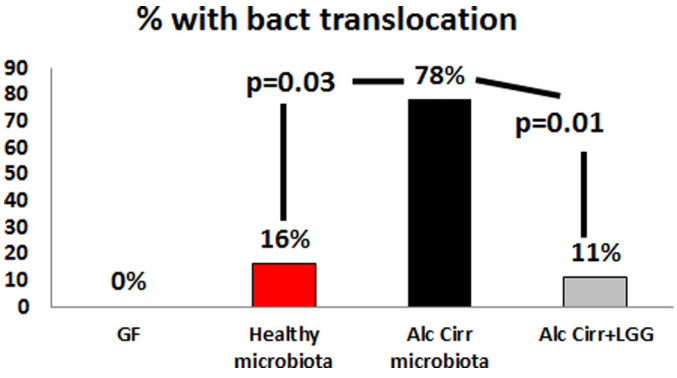
Background and Aims: A pro-inflammatory milieu with altered gut microbiota exist in alcoholic cirrhosis however, the contribution of the microbiota versus liver disease on this milieu is unclear. *Aims:* (1) impact of humanization with alcoholic cirrhotic compared to healthy stool, on bacterial translocation, microbial content/function and inflammation in germ-free (GF) mice and (2) changes with lactobacillus GG (LGG).
Methods: 4 gps of 10wk-old C57BL/6 GF mice were studied for 30 days; *Gp 1*: GF, *Gp 2*: humanized with healthy human stool, *Gp 3*: humanized with alcoholic cirrhotic stool, *Gp 4*: half of Gp 3 received LGG for last 15 days. At day 30, intestines, mesenteric lymph nodes (MLN), stool, blood, liver and brain were analyzed.

We analyzed microbiota in MLN (*bacterial translocation*), stool, intestines using multi-tagged sequencing. Microbial function was studied with cecal content metabolomics and stool bile acid (BA) profile. *Inflammation*: studied in liver, intestine, blood and brain. We compared all changes between Gps 1, 2 3 and between Gp 3 4.

Results: 6 mice each were in Gps 1 2 while 9 mice each were in Gps 3 4. *Cross-sectional analysis*: *Bacterial translocation*: was seen in 0 in Gp 1, 1/6 in Gp 2 and 7/9 in Gp 3 ($p = 0.03$, Figure). *Microbiota* in the stool and tissues showed dysbiosis in Gp 3 vs 2 (higher Porphyromonadaceae, Rikenellaceae and lower commensals: Lachnospiraceae, Ruminococcaceae and Clostridiales XIV). *Microbial function*: Gp 3 had higher fecal secondary BA deoxycholate (DCA) and significantly increased cecal content amino-acid metabolism, especially glutamine (100 fold) on metabolomics than Gp 2. *Inflammation:* higher endotoxemia (79 vs. 45 EU/ml, $p = 0.03$), cecal MCP-1 & IL-1 and cerebellar IL-6, TGR-5 & IL-1 seen in Gp 3 vs Gp 2. No liver inflammation was seen.

Before/after LGG: Bacterial translocation reduced (1/9 vs 7/9, $p = 0.01$) while stool/intestinal microbiota showed reduced dysbiosis (increased commensal taxa). *Microbial function*: DCA reduced significantly while, on metabolomics, LGG reduced glutamine by 80% and increased the anti-oxidant tyrosol by 56-fold. *Inflammation*: There was reduced endotoxemia (79 vs. 56 EU/ml, $p = 0.04$), cecal wall IL-6 & MCP-1 and cerebellar IL-1/IL-6 after LGG.

Conclusions: These results demonstrate that alcoholic cirrhosis-associated microbiota, but not healthy human microbiota, results in a pro-inflammatory milieu through dysbiosis and bacterial translocation, even without causing liver disease, and LGG supplementation may ameliorate these effects through altered anti-oxidant and glutamine metabolism.



O091

ORAL THERAPY WITH NON-ABSORBABLE CARBONS OF CONTROLLED POROSITY (YAQ-001) SELECTIVELY MODULATES STOOL MICROBIOME AND ITS FUNCTION AND THIS IS ASSOCIATED WITH RESTORATION OF IMMUNE FUNCTION AND INFLAMMASOME ACTIVATION

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Background and Aims: The host-microbiome interaction is pathological in cirrhosis promoting a dysregulated inflammatory response resulting in organ injury and diminished survival. Yaq-001 is an oral non-absorbable carbon shown to result in improvement in hepatic hemodynamics and markers of liver injury but the mechanism how it achieves these beneficial effect is unknown. The aims of this study were to determine whether the beneficial effects of Yaq-001 was related to the composition and associated functional state of the microbiome in bile-duct ligated cirrhotic animals.

Methods: BDL (n = 12) or sham (n = 12) animals were randomised to treatment with Yaq-001 (Yaqrit ltd. UK) from weeks 2–4. Analysis of urine samples was performed using ^{1}HNMRs. Bacterial DNA from stool was extracted, amplified and sequenced. Arterial plasma was co-incubated with HEK-Blue hTLR4 and IL-1β/IL-18 reporter cell lines. Constitutive ROS and LPS-induced ROS production from circulating monocyte and neutrophil populations was determined using flow cytometry.

Results: BDL was associated with a significant reduction in Peptidostreptococcaceae and Clostridium XI ($p < 0.05$) in stool compared to sham controls. Carbon therapy was associated with significant increases in firmicutes, in particular clostridia populations in stool with significant reductions in bacteroides populations ($p < 0.05$).

Functionally, BDL rats were found to have significantly higher urinary bile acids, trimethylamine oxidase, benzoate, glycine, acetate and lactate than sham controls ($p < 0.05$). Significantly lower levels of citrate, dimethylarginine and creatinine were observed in BDL compared to sham animals ($p < 0.05$). Carbon treatment results in a significant increase in urinary creatinine and bile acids and reduction in urinary glycine ($p < 0.05$).

A significant increase in IL18/IL1B expression was observed in untreated BDL rats compared to sham which was significantly attenuated with Yaq-001 ($p < 0.05$). Monocyte LPS-induced ROS production was significantly higher in BDL rats but attenuated with carbon treatment ($p < 0.05$).

Conclusions: The results of this study show that Yaq-001, which is a non-specific adsorbent has substantial effects of the composition and function of the microbiome in cirrhotic rats and positively modulates the function of neutrophils and monocytes reducing ROS production and inflammasome activation. These data provide compelling evidence that the gut bacterial products are important in mediating immune dysfunction of cirrhosis and is a target of therapy.

O092

MICROBIOLOGICAL ASSESSMENT OF ASCITIC FLUID IN LIVER DISEASE: CULTURE TECHNIQUES, SENSITIVITIES AND INTERPRETATION

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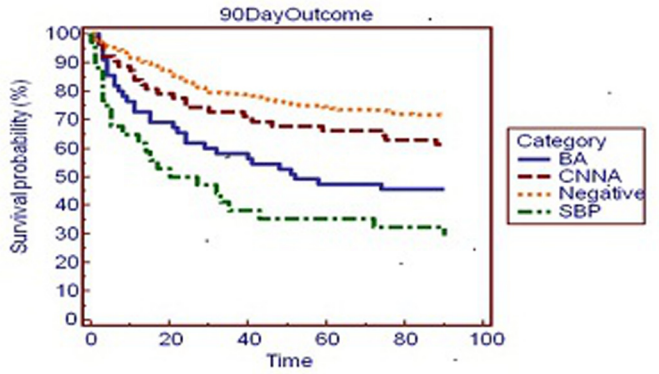
Background and Aims: Diagnostic paracentesis is a routine procedure for the assessment ascitic patients and the diagnosis of spontaneous bacterial peritonitis (SBP). It is recommended that samples are sent in blood culture bottles (BC) as well as universal containers (UC) for the best culture yield. However there is uncertainty regarding the significance of bacterascites (BA). We aimed to determine the culture yield of paired BC and UC samples and to assess outcome relative to the presence of SBP, BA or culture negative neutrocytic ascites (CNNA).

Methods: This was a retrospective review of all the ascitic fluid samples sent for microbiology analysis in North Glasgow between November 2010 and December 2013. Measured white cell count (WCC) and bacterial cultures were noted. Positive samples were defined as follows: **BA**, positive culture with WCC <250; **CNNA**, WCC >250 but negative culture; **SBP**, positive culture with WCC >250. Samples were classified on the basis of the initial sample or if >3 months since the previous sample.

Results: A total of 4131 samples were received from patients with liver disease. Of these there were 1520 BC and UC paired cultures in 503 individual patients; the majority having alcoholic

liver disease (74%). Pathogens were cultured in 118 paired samples: 47% only in BC; 34% in both BC and UC; 19% only in UC. Commensals were more common in UC: 72% compared with BC, 34%. 90 positive samples had had previous negative samples: 24 had negative samples less than 1 week prior to positive sample. 69% of pathogens cultured were sensitive to co-amoxiclav. BA and SBP were associated with a worse outcome compared with negative samples: HR 3.46 (2.0, 5.98); p < 0.0001 and HR 0.082 (0.039, 0.175); p < 0.0001 respectively. SBP, CNNA and BA samples had a worse 28 day outcome if the pre-ascitic tap C-reactive protein was >20: HR 0.476 (0.253, 0.895); p = 0.02. Culture of any pathogen was associated with a reduced 90 day survival compared with negative samples: HR 0.244 (0.159, 0.374); p < 0.0001.

Conclusions: Both BC and UC samples yield relevant pathogen cultures and both should be requested for the best chance of a positive culture. BA is not a benign finding. Direct culture of any pathogen irrespective of WCC is associated with a worse 90 day outcome.



O093

THE EMPIRICAL ANTIBIOTIC TREATMENT OF NOSOCOMIAL SPONTANEOUS BACTERIAL PERITONITIS IN PATIENTS WITH DECOMPENSATED LIVER CIRRHOSIS: RESULTS OF A RANDOMIZED CONTROLLED CLINICAL TRIAL

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Background and Aims: Spontaneous bacterial peritonitis (SBP) is a common and life-threatening complication of liver cirrhosis. Third generation cephalosporins are the first line empirical treatment of SBP. In recent years it has been observed an increasing rate of SBP due to third generation cephalosporins resistant bacteria, in particular in nosocomial SBP. Up to now a broader spectrum antibiotic regimen such as carbapenems and glicopeptides or lipopeptides have never been compared to third generation cephalosporins in the treatment of nosocomial SBP. The aim of our study was to compare the efficacy of meropenem plus daptomycin versus ceftazidime in the treatment of nosocomial SBP.

Methods: Consecutive patients with cirrhosis, ascites and nosocomial SBP were randomized to receive meropenem (1 g/8 hours) plus daptomycin (6 mg/kg/day) or ceftazidime (2 g/8 hours) plus albumin in both groups (1.5 g/kg on day 1 and 1 g/kg on day 3). A diagnostic paracentesis was performed after 48 h of antibiotic treatment. A reduction in ascitic fluid neutrophil count >25% of the pre-treatment value and/or isolation of bacteria resistant to the assigned treatment were considered a treatment failure and antibiotic therapy was changed accordingly. The primary outcome was the efficacy of the treatment defined by the resolution of SBP after 7 days of treatment.

Results: 32 patients were randomized and 31 were analyzed. The combination of meropenem plus daptomycin was significantly more effective than ceftazidime in the treatment of nosocomial SBP (86.7% vs 25%; p < 0.001). Third generation cephalosporin resistant bacteria and multidrug resistant bacteria were isolated in 81.3% and 37.5% of positive cultures, respectively. In the multivariate Cox regression analysis, ineffective response to first line treatment (hazard ratio [HR]=20.6; p = 0.01), development of acute kidney injury during hospitalization (HR = 23.2; p = 0.01) and baseline mean arterial pressure (HR = 0.92; p = 0.01) were found to be independent predictors of 90-day transplant free survival. The incidence of global adverse events (73.3 vs 81.3%; p = 0.68) and drug related adverse events (20.0 vs 25.0; p = 1.0) were similar between the two groups.

Conclusions: The combination of meropenem plus daptomycin is more effective than ceftazidime in the treatment of hospital acquired SBP. The efficacy of the first line treatment is associated with improved 90 days survival in these patients.
ClinicalTrials.gov Identifier: NCT01455246

O094

THE PRESENCE OF ECHOCARDIOGRAPHIC ABNORMALITIES INCREASES MORTALITY IN PATIENTS WITH SPONTANEOUS BACTERIAL PERITONITIS

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Background and Aims: Cirrhosis was found to be associated with echocardiographic abnormalities (ECHOABNs) such as left ventricular hypertrophy (LVH), systolic dysfunction (LVSD) and diastolic dysfunction (LVDD), as well as left atrial enlargement (LAE). Recent studies have highlighted the importance of the cardiac reserve for the adaptive response to acute circulatory stress in advanced cirrhosis, e.g. in patients with infections such as spontaneous bacterial peritonitis (SBP). We assessed the impact of abnormalities in cardiac structure and function in routine echocardiography on mortality in patients with cirrhosis and ascites (A) without SBP and (B) after a SBP episode.

Methods: One hundred twenty-seven patients with cirrhosis who underwent their first paracentesis at the Medical University of Vienna and did not develop SBP during the follow-up (group: no-SBP), as well as 78 patients diagnosed with SBP (group: SBP) with available information on routine echocardiography were included in this retrospective cohort study. Cox models were calculated to investigate the effect of echocardiographic abnormalities on transplant-free survival (TFS) and adjusted for all factors showing a trend toward a statistically significant difference ($P \leq 0.1$) between the no-SBP and the SBP group.

Results: *Patient characteristics:* Child–Pugh stage A:3%, B:47%, C:50%; median MELD score 19.3 (interquartile range:8.87); varices:75%; previous variceal bleeding:18%; hepatocellular carcinoma:15%; non-selective beta-blocker treatment: 42%. The prevalence of significant ECHOABNs was: LVH: 20%; LVSD: 2%; LVDD: 16%; LAE: 18%. The proportion of patients with ECHOABNs in the SBP group (55%) was higher, when compared to no-SBP patients (37%, $P = 0.011$).
While the presence of ECHOABNs had no impact on TFS in the no-SBP group [hazard ratio (HR): 1.183, 95% confidence interval (95% CI): 0.711–1.969; $P = 0.776$], it was associated with

substantially increased mortality in the SBP group (HR: 1.914, 95% CI: 1.026–3.571; *P* = 0.041).
Conclusions: The prevalence of ECHOABNs in patients with cirrhosis and ascites is high and further increases in patients with more advanced stages of cirrhosis, such as patients with SBP. Development of SBP may reveal impairments in the adaptive response to acute circulatory stress due to latent cardiac dysfunction, as the presence of ECHOABNs was associated with increased mortality in this subgroup of patients.

O095
HIGH TRANSFERRIN SATURATION DURING MAINTENANCE VENESECTION THERAPY IN HFE HEMOCHROMATOSIS IS ASSOCIATED WITH INCREASED MORBIDITY REGARDLESS OF SERUM FERRITIN LEVELS

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Background and Aims: Current international guidelines on the management of maintenance venesection therapy in *HFE* hemochromatosis (HH) recommend to keep serum ferritin (FRT) between 50 and 100 μg/l regardless of transferrin saturation (SAT). Our aim was to assess the impact of elevated SAT during maintenance therapy on HH-related morbidity
Methods: C282Y homozygotes were included if they had at least 3 years of follow-up after achievement of low body iron stores and at least one FRT and SAT determinations available per year.
A self-administered questionnaire assessing the onset or worsening of HH-related morbidity during maintenance therapy was sent to patients. Main questions concerned fatigue, joint and liver symptoms, and quality of life based on the assessment of libido, ability to work and to practice sport. Decreased quality of life was defined by a positive answer at 2 or 3 of these 3 questions.
Areas under the curves of SAT and FRT throughout maintenance therapy were determined to obtain mean SAT (mSAT) and FRT (mFRT)
Results: 268 patients (57% men, 78% probands) were included. Mean follow-up was 13.5±6 years. A number of 29±15 FRT and SAT values were available during this period. Median mSAT and mFRT were 47.6% [40.2–53.2] and 38.9 μg/L [27.7–59.4] respectively, with 40% of patients having mSAT >50%. 31% of women and 35% of men with mFRT <50 μg/L had mSAT >50%. Correlation between mSAT and mFRT was weak but significant (rho = 0.28, p < 0.001).
At multivariate analysis: (1) mSAT >50% was associated with mFRT >50 μg/L, smoking, BMI <27, absence of tea consumption, serum transferrin <1.7 g/L, and follow-up >15 years; (2) mFRT >50 μg/L was associated with mSAT >50%, overweight, alcohol consumption, and BMI >27; (3) worsening of hand arthropathy was associated with mSAT >50%, arthropathy at diagnosis, serum transferrin <1.7 g/L and follow-up >15 years; (4) worsening of quality of life was associated with mSAT >50%, age at diagnosis >55, arthropathy at diagnosis, and serum ferritin >50 μg/L at the end of the initial iron removal treatment.
Conclusions: In HH patients on maintenance venesection therapy, high transferrin saturation – regardless of serum ferritin level – is associated with increased HH-related morbidity, especially worsening of hand arthropathy. Further controlled studies are required to assess the potential benefit of tuning maintenance therapy according to SAT.

Liver inflammation, regeneration and cancer

O096
MATRIX METALLOPROTEINASE-10 CONTRIBUTES TO HEPATOCELLULAR CARCINOMA DEVELOPMENT IN A NOVEL CROSSTALK WITH STROMAL DERIVED GROWTH FACTOR 1/C–X–C CHEMOKINE RECEPTOR 4 AXIS

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Background and Aims: Matrix metalloproteinases (MMPs) play an important role in wound healing during acute liver injury. However, in chronic liver damage MMPs may be involved in hepatocellular carcinoma (HCC) development, contributing to a pro-tumorigenic microenvironment. This microenvironment is characterized by hypoxia and reactive oxygen species (ROS) production. The chemokine (CXC) receptor 4 (CXCR4)/stromal derived factor 1 (SDF1) axis promotes HCC cell proliferation, motility, angiogenesis and metastasis. Here we have characterized the expression and function of MMP10 (stromelysin 2) in hepatocarcinogenesis, and identified a new functional crosstalk between MMP10 and the CXCR4/SDF1 axis in HCC cells.
Methods: Hepatic MMP10 and CXCR4 expression were examined in diethylnitrosamine (DEN)-induced hepatocarcinogenesis in mice. The role of MMP10 was evaluated in DEN-treated $Mmp10^{+/+}$ and $Mmp10^{-/-}$ mice. A HCC cell line (Huh7) stably expressing MMP10 was generated (Huh7-MMP10). The functional crosstalk between MMP10 and the CXCR4/SDF1 axis was evaluated in human HCC cells.
Results: Hepatocellular MMP10 expression was progressively induced during hepatocarcinogenesis. Compared to $Mmp10^{+/+}$ animals $Mmp10^{-/-}$ mice showed less HCC incidence, smaller histological lesions, reduced tumor microvascular density and less lung metastases. CXCR4 expression in $Mmp10^{-/-}$ mice livers was significantly lower than in $Mmp10^{+/+}$. Huh7-MMP10 cells had increased CXCR4 expression levels and enhanced migratory capacity than control Huh7 cells. Up-regulation of CXCR4 expression in Huh7-MMP10 cells was attenuated by the antioxidant N-acetyl cysteine. Pharmacological inhibition of CXCR4 significantly reduced Huh7-MMP10 cell migration. On the other hand, MMP10 gene expression was induced by hypoxia and this response was enhanced by the CXCR4 ligand SDF1. Hypoxia/SDF1 triggered MMP10 expression through the MEK/ERK1/2 signaling pathway involving an AP-1 site in the proximal *MMP10* gene promoter.
Conclusions: To our knowledge this is the first report addressing the role of a MMP in hepatocarcinogenesis in the corresponding knockout mouse. MMP10 plays an important part in HCC development, participating in tumor angiogenesis, growth and dissemination. We have identified a new functional and reciprocal crosstalk between MMP10 and the CXCR4/SDF1 axis that can contribute to HCC progression and metastasis.

O097
MUTATION OF RELA THR505 ENHANCES LIVER REGENERATION FOLLOWING PARTIAL HEPATECTOMY

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Background and Aims: NF-κB is a family of transcription factors that are key regulators of the immune and inflammatory responses. NF-κB regulation is complex and occurs at many levels, including direct post-translational modifications (PTMs) of the subunits. Phosphorylation of RelA (p65) at Thr505 regulates proliferation, migration and autophagy in *in vitro* cellular systems; however its role and relevance in an *in vivo* system still remains unclear. Thus, the aim of this study was to analyse the role of RelA phosphorylation in vivo by mutating this site through creating a knock in mouse.
Methods: Knock in mutant mice where generated where Thr505 was substituted by Alanine, thus preventing phosphorylation. Partial hepatectomy (PhX) and acute CCl_4 administration was performed in WT and knock in mice. Animals were culled at 36, 72 hours and 5 days or at 24, 48 and 72 hours respectively. Paraffin stainings for BrdU and PCNA were used as markers of proliferation and γH2AX for DNA damage. Western blot was used to determined p53 and p-JNK expression.
Results: Thr505 mutation of RelA resulted in an increased liver to body weight ratio after PhX at 36, 72 hours and 5 days. Hepatocyte proliferation was increased after both injuries, PhX and acute CCl_4, at all the time points in RelA T505 mice versus WT, as assessed by PCNA and/or BrdU staining. Furthermore, mitotic body counts were also higher in RelA T505 mice after both injuries. Of note, proliferation after PhX was sustained for longer in RelA T505 mice, pointing towards an inefficient resolution of the proliferative response. Increased DNA damage, as determined by γH2AX staining, was seen in both WT and RelA T505 after 36 hours of PhX. However, the DNA damage was significantly higher in RelA T505 mice in comparison with WT. Finally, western blot analysis of these samples revealed increased expression of the p53 tumour suppressor and active JNK in RelA T505 mice.
Conclusions: RelA Thr505 phosphorylation provides an important and novel regulatory mechanism to control the proliferative response and prevent genomic damage in the liver.

O098
LONG TERM NLRP3 INFLAMMASOME ACTIVATION LEADS TO SEVERE LIVER FIBROSIS VIA INFLAMMATORY MACROPHAGE POLARIZATION AND DIRECT ACTIVATION OF HEPATIC STELLATE CELLS

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Background and Aims: The NLRP3 inflammasome, a caspase-1 activation platform critical for the processing of key pro-inflammatory cytokines has been implicated in the development acute and chronic liver diseases. However, the source and mechanisms of inflammasome mediated liver damage remains poorly understood. Therefore, our *aim* was to test the hypothesis that chronic NLRP3 inflammasome activation is a central mechanism for innate immune activation and the progression of liver disease.
Methods: *Nlrp3*^{D301NneoR} knock-in mice on a C57BL/6 background were crossed to C57BL/6.Cg-Tg(*Cre*/Esr1)–Estrogen Receptor Cre (CreT) mice to induce temporal *in vivo* expression of mutant NLRP3 in adult mice through administration of tamoxifen. After 16 weeks of inflammasome activation, mice were sacrificed and liver tissue and serum were harvested. To dissect cell specific effects of inflammasome activation, primary hepatic cells – hepatocytes, macrophages, and hepatic stellate cells (HSC) – were isolated from *Nlrp3*^{D301NneoR} CreT mice and activation of the NLRP3 inflammasome was induced *in vitro* in cultured cells via 4OH-tamoxifen treatment.
Results: Activation of the Nlrp3 inflammasome in Nlrp3^{KI} CreT over 16 weeks resulted in hepatomegaly while tamoxifen-injected control mice showed regular liver weights (liver to body weight % – 6.1±0.32% vs. 4.6±0.03%, $p < 0.05$). Analysis of liver histology revealed increased inflammation with neutrophilic infiltration and increased mRNA levels of myeloperoxidase (fold-change to WT 52.8±13.75, $p < 0.05$). Liver sections of *Nlrp3* mutant mice displayed significantly increased collagen deposition and expression of TIMP1 (fold-change to WT 7.5, $p < 0.05$) when compared to tamoxifen-injected controls. Activation of NLRP3 in isolated hepatocytes resulted in a 2-fold increase in the expression of proliferating cell nuclear antigen (PCNA). Studies on isolated hepatic macrophages revealed inflammatory polarization upon NLRP3 activation with elevated expression levels of iNOS, pro-IL-1β, and TNF-α, while levels of arginase 1 were reduced. Moreover, we found that activation of the NLRP3 inflammasome in isolated HSC resulted in increased expression of α-SMA and tissue inhibitor of metalloproteinase-1 (TIMP-1).
Conclusions: Our study uncovers a crucial role for the NLPR3-inflammasome in the development of liver injury and fibrosis and provides unique models to assess novel therapeutic strategies aimed at halting the progression of chronic liver diseases.

O099
CYSTEINE CATHEPSINS CONTROL LIVER INFLAMMATION THROUGH REGULATION OF SIRTUIN-1 ACTION ON P65-NFκB SUBUNIT

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Background and Aims: Sirtuin-1 (SIRT1) is a nuclear protein deacetylase that controls hepatic lipid metabolism in hepatocytes and modulates the acetylation status of the RelA/p65 subunit of NFκB, thus playing a pivotal role in regulating the inflammatory, and apoptotic responses in the liver. Cysteine cathepsins were originally believed to participate exclusively in terminal protein degradation during necrotic and autophagic cell death. However, nowadays it is well established that they execute numerous specific functions participating in important physiological processes, such as MHC-II-mediated antigen presentation, pro-hormones activation, and others. To our knowledge, however, the potential participation of cysteine cathepsins (such as Cathepsin B, CtsB or S, CtsS) on SIRT1 regulation of inflammation in the liver has not been reported, and is the aim of this study.
Methods: Hepatocytes (HC), Kupffer cells (KC) and hepatic stellate cells (HSCs) were isolated and cultured in plastic. Human LX2 activated HSCs, RAW264.7 macrophages, and Hep3B cells were also used. Cells were exposed to LPS (50 ng/ml) or TNF (50 ng/ml), in the presence of CtsB or CtsS inhibitors (Ca074Me, 25μM and Z-FL, 10μM). SIRT1, p65 (NF-κB), α-SMA, β-actin, Lamin A/C, among others, were evaluated by western blotting. κB-dependent genes (A20, MCP1, and IL6) were detected by RT-qPCR.
Results: SIRT1 levels increased during the activation of primary mouse HSCs (from days 1 to 8) paralleling CtsS, CtsB, and α-SMA expression. Challenging HSCs or LX2 cells with TNF or LPS resulted in SIRT1 downregulation followed by p65-NFκB nuclear translocation, and enhanced κB-dependent gene expression (MCP1 and IL-6). In this setting, CtsB or CtsS inhibition resulted

in decreased p65 nuclear translocation and κB-dependent gene expression, which was accompanied by enhanced SIRT1 expression. Similar results were observed in murine primary Kupffer cells and hepatocytes, and were replicated in RAW264.7 and Hep3B cells. In addition, *in vivo* LPS challenge in mice was accompanied by enhanced expression of MCP1 and IL6 mRNA, which was greatly reduced after CtsB or CtsS inhibition. Thus indicating that inhibiting cysteine cathepsins limits hepatic inflammation through the regulation of SIRT1 deacetylation of p65-NFκB and down-regulation of κB-dependent gene expression.

Conclusions: Cysteine cathepsins modulate liver inflammation by controlling SIRT1-dependent deacetylation of p65-NFκB subunit.

O100

THE HEPATIC MICROENVIRONMENT INDUCES A CSC PHENOTYPE AND DETERMINES THE PROGNOSIS OF HCC PATIENTS

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Background and Aims: The cancer stem cell (CSC) hypothesis is an emerging concept in cancer research that provides a plausible explanation for the observed phenotypic heterogeneity of many cancers including hepatocellular carcinoma (HCC). However, despite profound therapeutic implications the prognostic relevance of CSCs and their cellular localization within the tumor formation remain controversial.

Methods: Expression levels and localization of established CSC markers were assessed in pre-neoplastic lesions as well as 30 HCCs using qRT-PCR, imunohistochemistry and Western-Blotting. Integrative whole genome and transcriptome analyses of different tumor regions as well as tumor-surrounding liver (SL) were performed to identify associated molecular alterations and integrated with our existing HCC database.

Results: Expression patterns of established CSC markers were surprisingly heterogeneous. While classical markers such as GPC3 were induced in tumor tissue, activation of CSCs was predominantly observed in SL and continuously decreased from pre-neoplastic lesions to HCC. Consistently, genomic and transcriptomic profiles between SL and different tumor regions were quite distinct. Progressive increase in genetic alterations and activation of pathways related to proliferation as well as apoptosis was observed in the tumor tissue, while the invasive tumor margin (TM) was characterized by inflammatory and EMT-related gene sets as well as activation of pro-survival signaling such as ERK and FOS. Consistently, integration of the different signatures with our database of 53 HCC revealed that the TM signature was associated with the survival of HCC patients.

Conclusions: CSCs in HCC are heterogeneous. The CSC phenotype is predominantly determined by the permissive tumor microenvironment. However, pro-oncogenic properties might originate in the TM. The activation of key oncogenic features as well as immune-response signaling indicates that the cross-talk between tumor and microenvironment might be a promising therapeutic and/or preventive target.

O101

HISTONE VARIANT macroH2A1 ORCHESTRATES ESCAPE FROM HEPATOCYTE SENESCENCE DURING AGEING AND CANCER

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Background and Aims: The epigenetic basis of age-associated progression of liver diseases towards hepatocellular carcinoma (HCC) is unclear. MacroH2A1 is a variant of histone H2A1, present in the two isoforms macroH2A1.1 and macroH2A1.2, with fundamental roles in cell homeostasis. MacroH2A1 protein levels are upregulated in human HCC. MacroH2A1 is also marker of senescence associated heterochromatic foci (SAHF) and synergizes with DNA demethylating agent 5-aza-2′-deoxycytidine (5-aza-dC) in silencing tumor suppressor genes in human fibroblasts. Its role in cell senescence during HCC is unknown.

Methods: We used liver tissues from aged wild type mice, naked mole rats and human HCC to analyze macroH2A1 levels during hepatocyte senescence. Stable HepG2 cell line overexpressing macroH2A1.1 and macroH2A1.2 tagged with GFP, HepG2 cells stably silenced (knock-down) for macroH2A1 and macroH2A1 knock-out mice were generated for biochemical studies and to study the effects of macroH2A1 on 5-aza-dC induced cell senescence by B-gal assay, quantitative RT-PCR, chromatin immunoprecipitation sequencing (ChIP-Seq) and RNA sequencing (RNA-Seq). Cell cycle was assessed by FACS analyses, while cell proliferation and migration was evaluated by MTT and scratch assay, respectively. SAHF were analyzed by confocal microscopy.

Results: We show that protein levels of macroH2A1 isoforms are increased in the livers of old rodent and humans, and in HCC tissue of both young and old age. Human HCC HepG2 cells overexpressing macroH2A1 escape 5-aza-dC-induced senescence, as determined by cell proliferation/migration/cell cycle assays, B-gal staining and SAHF marker HP-1 cellular localization, and exhibit a synergistic global DNA hypomethylation. Deletion of macroH2A1 in HepG2 cells and in old mice had opposite, pro-senescence, effects as determined by cell cycle assays and hepatic X-gal staining, respectively. System analysis of ChIP-Seq combined with RNA-Seq data in HepG2 cells revealed complex macroH2A1 genome occupancy correlated with anti-senescence transcriptional patterns.

Conclusions: Together with our previous report indicating both macroH2A1 isoforms as HCC markers (Rappa F et al., PLOS ONE 2013), our data demonstrate that both macroH2A1 isoforms are oncogenic in the liver. In fact, they allow HCC cells to escape senescence induced by a chemotherapy drug, increasing their aggressiveness. We propose macroH2A1 as a strong candidate target for epigenetic- and senescence-based HCC chemotherapy.

O102

GALECTIN-1 EXPRESSION IS ESSENTIAL FOR AN EFFECTIVE LIVER REGENERATION

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Background and Aims: Galectin-1 (Gal1) protein is encoded by the *Lgals1* gene and acts both extracellularly and intracellularly as an immune/inflammatory regulator, modulating innate and adaptive immune responses. In the liver, it is expressed mainly by non-parenchymal cells and acts mostly as an inducible anti-inflammatory regulator. Here, we explored the role of Gal1 in liver regeneration (LR) using a murine model.

Methods: We performed 70% partial hepatectomy (PHx) in the C57BL/6 WT and Gal1-knockout (Gal1-KO, *Lgals1*$^{-/-}$) mice and monitored the process of LR in both strains at 2, 6, 24, 48, 72 and 96 hours following operation. Expression of several known PHx-induced genes was monitored by RT-PCR at 2, 6 and 24 hours following operation. Expression of selected proteins was validated by immunohistochemistry and immunoblotting.

Results: We demonstrate an early Gal1 induction in the liver tissue of WT mice upon PHx, and report a significant delay in LR following PHx in the Gal1-KO compared to the WT liver. This delay was accompanied by a decreased monocyte/macrophage recruitment; a decreased level of nuclear cyclin D1 protein in all liver cells analyzed, and by an increased level of hepatocyte nuclear p21 protein in the Gal1-KO versus WT livers at 24 and 48 hours following PHx. Transcriptional analysis revealed a significantly decreased expression of the *Il6* and *Atf3*, both indispensable for LR, at two hours post-PHx, and a significantly increased expression of the anti-inflammatory *Tnfaip3* and *Zfp36* genes at six hours post-PHx, in Gal1-KO compared to WT livers. Transient steatosis, which is imperative for LR following PHx, was significantly delayed and decreased in the Gal1-KO compared to the WT liver. This was accompanied by a significantly decreased expression in the mutant liver of several genes encoding lipid metabolism regulators.

Conclusions: Our findings reveal for the first time that the Gal1 protein is essential for efficient LR following PHx through its known regulatory activities on liver inflammation and cell proliferation, and possibly through its newly discovered regulation of lipid storage in the regenerating liver.

O103

METASTASIS DEVELOPMENT IN A NOVEL MOUSE MODEL OF ADVANCED LIVER CANCER

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Background and Aims: Liver cancer is one of the leading causes of cancer related mortality worldwide. With limited treatment options, especially in the advanced stages of the disease, the development of novel therapies is urgently needed. The absence of reliable mouse models has been a major limitation in the study of advanced, metastatic liver cancer in the past. Here, we present a novel genetically engineered mouse model of primary liver cancer. Combining expression of oncogenic Kras with inactivation of the tumour suppressors RB and p53 we mimic molecular events that occur in the majority of human liver cancers and achieve rapid formation of hepatic tumours with extrahepatic metastases in our mice.

Methods: Adult *Rb*$^{lox/lox}$;*p53*$^{lox/lox}$;*Kras*$^{LSL-G12D/+}$ (RPK) mice were injected intravenously with liver specific AAV-Cre. Upon liver tumour formation, individual lesions were microdissected and analysed. mRNA microarray analysis was performed on an Affymetrix platform and validated using qPCR. Protein expression was visualized by immunohistochemistry and SDS page gel electrophoresis.

Results: The RPK mouse model combines liver specific inactivation of RB and p53 with activation of Ras signalling. After gene recombination, RPK mice rapidly develop hepatic tumours within 3 to 4 months. Cancer lesions in this mouse model express hepatocytic (AFP$^+$ and/or ALB$^+$) as well as biliary (CK19$^+$) differentiation markers. Comparison of gene expression microarrays from RPK tumours with data from human liver cancer samples confirmed clustering with human un-differentiated hepatocellular carcinomas and cholangiocarcinoma. Interestingly, selective targeting of hepatocytes in RPK mice showed that liver tumours of mixed or even biliary differentiation arise from mature hepatocytes. On the molecular level, RPK liver tumours showed activated ERK and AKT signalling downstream of Kras, as well as expression of metastasis associated genes. Strikingly, extrahepatic metastases to the abdominal lymph nodes or lung were found in the majority of mice with advanced tumour lesions.

Conclusions: The RPK mouse model presents a novel platform to study key molecular mechanisms in liver carcinogenesis. Importantly, the highly consistent occurrence of metastatic lesions gives the unique opportunity to investigate molecular drivers of metastasis development in vivo. The RPK model will help to answer important question in liver tumour biology and aims to identify novel targets in the treatment of human liver cancer.

Liver transplantation

O104

PREDICTIVE MODEL FOR THE NEED FOR LIVER TRANSPLANTATION IN SYMPTOMATIC POLYCYSTIC LIVER DISEASE

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Background and Aims: Polycystic liver disease (PCLD) with massive hepatomegaly can become an indication for liver transplantation (LTx). To prioritize these patients on the LTx-waiting list, exception criteria based on simple, objective and highly reproducible factors are needed. We aimed to explore the parameters contributing to the severity of the PCLD disease which could be used in the decision/prioritization for LTx.

Methods: We performed a large retrospective cohort analysis of PCLD patients referred to 5 European tertiary centers. Existing

clinical and abdominal scan data were re-analysed for liver volume and index (estimates how many times the liver has been enlarged), stage of malnutrition (sarcopenia), and extra-hepatic and local cyst complications.

Results: The study population consisted of 216 patients: 87% (n = 187) were women, 83% (n = 180) had ADPKD and 36% (n = 67) suffered from sarcopenia. 137 patients (63.4%) were deemed eligible for LTx based on clinical judgement of the transplant teams; of them 50 (36%) received a combined liver and kidney transplantation (cLTKTx). The indication for LTx was severe hepatomegaly related complaints in 85% (n = 116). In patients with a previous kidney transplantation the most frequent indication for LTx (47%) was recurrent liver cyst infection. Factors which predicted the need for LTx (PCLD severity score) were: liver volume index >5, serum albumin <4.1 g/l, age <47 yrs and the presence of ascites. A multivariable model combining these factors had an area under the curve of 0.81 (SE: 0.05; 95% CI: 0.72 to 0.88, p < 0.0001) and correctly classified 92% of the patients. In cLTKTx patients, a creatinine clearance <32 ml/min/1.73m^2 correctly classified 92% of the patients with the need for KTx.

Conclusions: In patients with PCLD, factors which can be assessed only by one scan (liver volume, nutritional stage, ascites) provide objective information that could be used for the decision/prioritization for LTx. In patients with kidney transplantation, recurrent liver cyst infections is also an indication for LTx.

O105

COMPARISON OF SHORT-TERM AND LONG-TERM OUTCOMES AFTER DCD AND DBD DONOR LIVER TRANSPLANTATION

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Background and Aims: The increasing demand for suitable organs for liver transplantation (LT) has expanded the use of donation after cardiac death (DCD) allografts, despite evidence of higher incidence of primary non-function (PNF) and primary ischaemic cholangiopathy (PIC) with these grafts. To evaluate our experience, we compared the outcome of LT with donation after brain death (DBD) and DCD grafts in a large single centre cohort.

Methods: The outcomes of 293 controlled DCD liver transplants were compared to 293 matched DBD controls, performed at a single institution over a 15 year time period. DCD livers were allocated to adult patients with no previous major abdominal surgery. There were no age cut-offs for DCD grafts, but warm ischaemic time (WIT) was limited to 30 minutes or less.

Results: There was no difference in gender and recipient's BMI between DBD and DCD transplants, however DBD donors were older and had longer cold ischaemic times (CIT). Patients that received DCD grafts had higher levels of post-transplant serum AST ($p < 0.0005$), however, there was no significant difference in duration of intensive care unit or hospital stay for recipients of either type of allograft. PNF was significantly higher in DCD allografts ($p < 0.0005$), nevertheless there were no statistically significant differences in the incidence of renal replacement therapy usage, rejection episodes, vascular or biliary complications, including PIC, between the 2 groups. Although more patients with hepatocellular carcinoma (HCC) were allocated a DCD graft, there was no difference in HCC recurrence. Overall patient and graft survival was higher in the DBD group (p = 0.025, p = 0.002) but when transplants for HCC were excluded, no difference in overall patient and graft survival were observed.

Conclusions: The data shows a favourable outcome of LT using DCD allografts with no increase in biliary or vascular complications. Minimising CIT and optimising donor/recipient matching are crucial in order to achieve good outcome. More work is required to explore the impact of DCD status on HCC recurrence.

O106

THERAPEUTIC PLASMA EXCHANGE MODULATES INNATE IMMUNE ACTIVATION AND IMPROVES OUTCOME IN PATIENTS WITH ACUTE LIVER FAILURE

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Background and Aims: Acute liver failure (ALF) involves a mortality of >40% despite transplantation. Overwhelming hepatocyte death induces a systemic inflammatory response (SIRS) and confers adverse outcome. Monocytes are key effectors of SIRS in the pathogenesis of ALF. A multicentre study evaluating therapeutic plasma exchange (TPE) demonstrated a 20% survival benefit. TPE has been established for various immunologically driven disorders. Recent data highlight its ability to dampen innate inflammatory responses. We evaluated the effect of TPE on phenotype and function of immune effector cells.

Methods: Plasma samples from ALF patients admitted to Rigshospitalet (n = 11) and King's College Hospital (n = 9) who underwent TPE were taken before and after the first exchange. Healthy peripheral blood mononuclear cells were cultured for 24 hours in medium containing 25% plasma from ALF patients. The effect of plasma obtained before/after TPE was compared to plasma from patients who did not receive any therapeutic intervention (natural course, n = 11). Immunophenotyping (CD14, CD16, HLA-DR, CD86, MERTK, CD163, CD64, CCR7, AnnexinV) and TNF-α/IL-6 production of monocytes in response to lipopolysaccharide (LPS) and phenotyping of lymphocytes (CD4, CD8, CD56, CD127, CD25, FoxP3) and LPS-induced IFN-γ/IL-2/TGF-β/IL-10 production were assessed by flow cytometry.

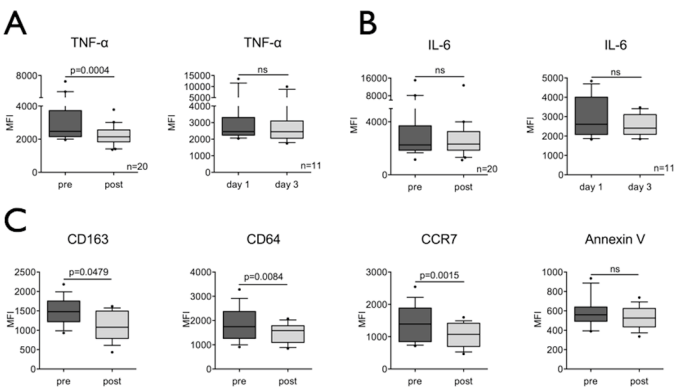

Figure: (A) Monocytes TNFα production in response to lipopolysaccharide in ALF patients pre- and post-TPE (left) and without therapeutic intervention (right). (B) Monocyte IL-6 production. (C) Reduced inflammatory activation markers on monocytes pre- and post-TPE (CD163, CD64, CCR7) and Annexin V staining excluding apoptosis.

Results: Across TPE, a significant reduction of disease severity markers such as MELD (p = 0.0156), lactate (p = 0.0078) and AST (p = 0.0078) was observed. Monocyte counts (p = 0.8125) and apoptosis rates (AnnexinV; p = 0.1354) before and after TPE were comparable. Monocyte pro-inflammatory response (TNF-α) to LPS

was significantly reduced in plasma post- compared to pre-TPE ($p = 0.004$; CD14+16-:$p = 0.0003$/CD14+16+:$p = 0.0014$), there was no change following natural course of disease. IL-6 production was unaffected. Immunophenotyping of monocytes in post TPE plasma revealed reduced expression of activation markers CD64 ($p = 0.0084$), CCR7 ($p = 0.0015$) and CD163 ($p = 0.0479$), but no changes following natural course. Numbers of CD4/CD8, regulatory T-cells, NK-cells were unchanged, however CD4-cell secretion of IL-10 ($p = 0.0144$) and TGF-β ($p = 0.0332$) was significantly reduced.
Conclusions: Clearance of inflammatory mediators from the plasma by TPE reduces disease severity, SIRS activation and extrahepatic organ dysfunction through suppression of innate immune cell activation in ALF; providing a mechanistic explanation for the beneficial effect of TPE on outcome in ALF.

O107

IL-22 SECRETION IS REQUIRED FOR LIVER REGENERATION AND IS MODULATED BY EXTRACELLULAR NUCLEOTIDES

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Background and Aims: Liver regeneration after hepatic resection is a complex and highly coordinated process between various cell types including innate immune cells. Nucleotides, such as ATP are released by such cellular injury bind to specific purinergic receptors expressed and further modulate effector functions of various immune cells including cytokine secretion. The cytokine IL-22 exhibits specific hepatoprotective and proliferative properties in various models of liver injury and repair.
Methods: Partial hepatectomy is performed in mice null for IL-22 and CD39 and assessed using PCNA and Ki67 staining. Cytokine sercretion ex vivo was assessed in response to IL-23 or PMA and ionomycin
Results: Serum levels of IL-22 are elevated at early time points post partial hepatectomy. Specific fractions of IL-22 producing cells are shown in the liver that include innate lymphoid cells like conventional NK cells, Rorgt positive ILC3 cells and other cells fractions include NKT cells, T cells and lineage negative cells. Further, IL-22 is required for optimal liver regeneration as hepatocellular proliferation was assessed by Ki67 and PCNA staining was significantly delayed and liver injury was increased in mice null for IL-22 compared to wild type mice. Interestingly, cell fractions that produce IL-22 express CD39 a major vascular ectonucleotidase that hydrolyzes extracellular ATP to ADP and AMP. In CD39 null mice NK cells, CD4 T cells, NKT cells, Rorgt positive ILC3 cells and lineage negative cells exhibit lower secretion of IL-22 in response to IL-23 or PMA/Ionomycin. In vivo secretion of IL-22 post partial hepatectomy was significantly reduced in mice lacking CD39 compare to wild type mice. Dysregulated activation of P2 type receptors in response to extracellular nucleotides was explored using P2 receptor agonists. Administration of ATPgammaS, ADPbetaS (non-hydrolysable ATP analogs) stimulates IL-22 expression that is not dependent on IL-23 stimulation. Further IL-23 dependent secretion of IL-22 is modulated by extracellular ATP. These findings were substantiated in vivo by showing that elevated hydrolysis of extracellular ATP by soluble ectonucleotidase apyrase is associated with significant decrease of IL-22 expression in response to IL-23.
Conclusions: We show that IL-22 secretion is required for liver regeneration and that the secretion of IL-22 by various subsets of hepatic immune cells is modulated by CD39 and extracellular nucleotides.

O108

RESIDUAL HCV-RNA IN LIVER EXPLANTS FROM PATIENTS UNDERGOING SOFOSBUVIR AND RIBAVIRIN TREATMENT WHILE AWAITING LIVER TRANSPLANTATION IS NOT ASSOCIATED WITH HCV RELAPSE

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Background and Aims: Sofosbuvir (Sovaldi™, SOF) in combination with ribavirin (RBV) administered to HCV-infected patients with hepatocellular carcinoma awaiting liver transplant (LT) prevented recurrence of HCV post-LT in 70% in those individuals with undetectable serum HCV-RNA at time of LT (Curry et al Gastroenterology 2014). The present study examined the presence of HCV-RNA in available liver explants from patients in the Phase 2 clinical study.
Methods: Liver explant samples were collected from 38 HCV-infected cirrhotic patients that underwent LT. All patients received 3 to 52 weeks of SOF+RBV while awaiting LT. HCV-RNA levels in the liver were determined by quantitative real-time PCR in triplicate experiments. The limit of detection was 6 copies per reaction (S 86%, Sp 91%). Liver explants from 39 HCV-RNA negative patients were used as controls.
Results: Thirty-four patients were included in the final analysis. Twenty-three out of 34 (68%) patient liver explants were HCV-RNA positive (range 0.7–67566 copies/ug) and 11 (32%) were HCV-RNA negative. HCV-RNA was not detected in any of the control explants. Treatment duration and time with undetectable HCV-RNA in serum before LT were significantly lower in patients with HCV-RNA in liver explants (16 and 7 weeks, respectively), compared to those with undetectable HCV-RNA (24 and 13 weeks, $p = 0.037$ $p = 0.045$, respectively). Twenty-four out of 34 patients (71%) achieved SVR12 after LT and 10 patients (29%) presented recurrent HCV infection. Interestingly, HCV-RNA was detected in liver explants from 16 (68%) of the 24 responders and in 7 (70%) of the 10 non-responders ($p = 0.850$).
Conclusions: SOF+RBV regimen is an efficacious therapy for preventing HCV recurrence. The presence of HCV-RNA in liver explants did not significantly correlate with the likelihood of recurrent HCV infection post-LT. Residual HCV in liver explants may represent non-functional HCV-RNA (incomplete genomes versus unfit viral strains) in the context of HCV-RNA polymerase inhibition by SOF.

O109

TREATMENT OF SEVERE HCV-RECURRENCE AFTER LIVER TRANSPLANTATION USING SOFOSBUVIR-BASED REGIMENS: THE ANRS CO23 CUPILT STUDY

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Background and Aims: Recurrence of HCV after liver transplantation (LT) can rapidly lead to liver graft cirrhosis, and therefore graft failure and re-transplantation or death.

Study aim was to assess efficacy and tolerance of sofosbuvir (SOF)-based regimens for the treatment of HCV recurrence in patients with severe fibrosis after LT.

Methods: The ANRS CO23 CUPILT study is a prospective nationwide cohort including patients with HCV-recurrence following LT treated by second-wave direct antivirals. The present study focused on patients included between Oct 2013 and Feb 2014 and diagnosed with HCV recurrence and liver graft extensive fibrosis (METAVIR F3/F4). Treatment regimens were prescribed according investigator's discretion. Patients were followed at W0, W2, W4, W12 and W24.

Results: A sofosbuvir (SOF)-based regimen was administered to 35 patients fulfilling inclusion criteria. The median delay from LT was 65 months [29–111]. The characteristics of patients at W0 were: median age: 61 years [53–67]; male: 77%, G1: 77%, G2: 3%, G3: 14%, G4: 6%; ALT: 84 IU/L [43–126], gGT: 137 IU/L [58–290], bilirubin: 15.5 micromol/L [10–33], albumin: 37.7 g/L [31.1–40.6], median HCV RNA: 6.3 log IU/ml [5.8–6.6]. Ascites was present in 1 (3%) patient. Three (9%) were HIV-co-infected and 33 (94%) failed to previous antiviral therapy containing first generation protease inhibitors in 6 (17%) cases. The following regimens were used: Peg-IFNa + SOF + ribavirin (n = 3), SOF + ribavirin (n = 7), SOF + daclatasvir (n = 11) and SOF + daclatasvir + ribavirin (n = 14) for 24 weeks. All patients were alive without re-transplantation at W24. HCV RNA was not detectable at W2 and W4 in 3 (12%) and 5 (15%) patients, respectively. At W12, 6 (17%) patients had HCV RNA <15 IU/ml and 28 (80%) were not detectable. At W24, HCV RNA was not detectable in all patients. The rates of ALT and gGT normalization at W12 were 85% and 62%, respectively. Median bilirubin serum level decreased from 15.5 to 12.2 at W24. Albumin level increased from 37.7 to 39.8 g/L at W24. This was accompanied by clinical improvement including nutritional status (weight gain from 72.2 to 75.3 kg). Ascites was present at W24 in 2 patients. Severe adverse-events occurred in 11 (31%) patients before W24.

Conclusions: SOF-based regimens show very promising results regarding high rates of end of treatment virological response in patients with severe HCV recurrence after LT. Sustained virological response rates will be presented during the meeting.

O110

SIMEPREVIR + SOFOSBUVIR COMBINATION THERAPY FOR RECURRENT GENOTYPE-1 HEPATITIS C IN LIVER TRANSPLANT RECIPIENTS: A REAL-LIFE MULTICENTER EXPERIENCE

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Background and Aims: Recurrent hepatitis C (HCV) after liver transplantation (LT) remains a major cause of morbidity and mortality. Data on the safety and effectiveness of simeprevir + sofosbuvir (SMV+SOF) therapy in "real-life" patients with HCV after LT are limited.

Aim: To evaluate the safety and effectiveness of SMV+SOF therapy in patients with genotype (GT)-1 HCV recurrence after LT.

Methods: All GT-1 HCV patients who underwent LT and received, or are receiving, SMV+SOF for 12–24 weeks were retrospectively identified at 4 centers. Treatment response by HCV RNA during therapy and at weeks 4 and 12 after completion of therapy (sustained virologic response, SVR4 and SVR12, respectively) were determined. Adverse events (AE), significant AE (SAE), changes to immunosuppression and survival were recorded.

Results: 40 patients were included in the study, 82% male, 62.5% Caucasian, with a mean age of 61.3±6.2 years; 60% had GT-1a, 35% had GT-1b and 5% had no subtype. The median time from LT to SMV+SOF initiation was 991 days (44–4563 days). 63% had F0–2 fibrosis, and 37% had F3–4 fibrosis. 90% were on tacrolimus. All patients (n = 22) who completed 12 weeks of therapy achieved undetectable HCV RNA at this time point. SVR4 was achieved in 77% (10/13). There were 2 relapses after completion of 12 weeks of SMV+SOF, and one patient truncated therapy at 6 weeks due to progression of hepatic decompensation and later died. Adverse events were reported in 53% of patients with the most common: constitutional (42%), anemia (10%), and gastrointestinal symptoms (10%). There were 4 SAEs consisting of syncope due to dehydration (n = 1), renal insufficiency (n = 2), and progressive hepatic decompensation leading to death (n = 1). There were no significant changes required on immunosuppression while on therapy.

Conclusions: SMV+SOF is an effective regimen for the treatment of HCV recurrence after LT, with no significant impact on dosing of immunosuppression. Adverse events were manageable in almost all patients, with SAEs being uncommon. However, given the rapidly evolving treatment landscape of HCV, the future utility of SMV+SOF in LT recipients with hepatic decompensation from recurrent HCV appears uncertain.

O111

OPERATIONAL TOLERANCE CAUSES A LONG LASTING ACTIVE IMMUNOREGULATION WITHIN THE GRAFT

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Background and Aims: Immunosuppression (IS) can be discontinued from selected, stable patients after liver transplantation resulting in operational tolerance (OT). While biomarkers can predict the outcome of IS withdrawal, the mechanisms mediating OT remain elusive.

Methods: In the current study, we analyzed serial liver biopsies obtained from adult liver recipients enrolled in a prospective multi-center IS withdrawal trial employing immunophenotyping and transcriptional profiling. Liver samples were collected before the initiation of IS withdrawal, at the time of rejection, or 1 and 3 years after complete drug discontinuation. In parallel immune cells from peripheral blood were analysed by flow analysis.

Results: Out of the 102 recipients participating in the trial, IS withdrawal was successful in 41 recipients. We analyzed mechanisms of tolerance in 15 patients with serial biopsies. The number of liver infiltrating T cell subsets did not differ at baseline between patients who rejected and those who successfully discontinued IS, However, to our surprise the tolerated grafts exhibited portal tract expansion with increased T cell infiltration despite normal transaminases and no signs of rejection one year after IS withdrawal. This was associated with preferential accumulation of CD4+Foxp3+ T cells, a shift in the CD4/CD8 T cell ratio and a trend towards up-regulation of immune activation and regulatory genes. At three years after induction of operational tolerance the grafts had still large inflammatory infiltrates, but with reduced CD8+ T cells leading to an increased CD4/CD8 ratio suggestive of additional deletional mechanisms. The inflammatory gene signature returned to baseline 3 years after IS withdrawal. Changes within the graft were not paralleled by analysis of PBMCs

Conclusions: We report here for the first time data suggesting that in human liver transplant recipients OT is an active, long-lasting phenomenon in which IS withdrawal elicits dominant immunoregulatory mechanisms that restrain effector alloimmune

responses. The results will need to be taken into account when designing future diagnostic and therapeutic clinical studies aiming at achieving allograft tolerance in clinical organ transplantation.

Viral hepatitis B & D: Clinical

O112

HBsAg CLEARANCE AFTER ADDITION OF 48 WEEKS OF PEGIFN IN HBeAg NEGATIVE CHB PATIENTS ON NUCLEOS(T)IDE THERAPY WITH UNDETECTABLE HBV DNA FOR AT LEAST ONE YEAR: FINAL RESULTS FROM ANRS-HB06 PEGAN STUDY: MULTICENTER RANDOMIZED CONTROLLED PHASE III TRIAL

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Background and Aims: Uncontrolled studies suggest that addition of PEGIFN in CHB patients receiving NUCs with undetectable serum HBV DNA may increase HBsAg clearance. We conducted a multicenter randomized controlled study to evaluate this strategy.
Methods: The key inclusion criteria were: HBeAg negative CHB and documented negative HBV DNA while on stable NUC regimens for at least 1 year. Patients with PEGIFN contra-indications were excluded.
From January 2011 to July 2012, 183 patients [86% male, mean age 47.6 years (range 28–74), HBV DNA undetectable for 198 weeks (range 13–1074)], were randomized to receive a 48 weeks course of 180 μg/w PEGIFN-alfa-2a (Pegasys) in addition to the backbone NUC regimens (Group 1: n = 90) or no additional therapy (Group 2: n = 93). Patients were stratified according to the HBsAg titers (< or ≥2.25 log IU/ml). NUC regimens remained unchanged during the study period up to week 144. Treatments discontinuation was allowed if HBsAg clearance was sustained for 24 weeks. Patients were seen monthly during the first 48 weeks, then every 3 months.
The primary end point was the proportion of patients with serum HBsAg clearance at week 96. Secondary endpoints included HBsAg clearance at week 48.
Results: 85 patients initiated PEGIFN in group 1, 17 patients discontinued prematurely PEGIFN due to adverse events, and 4 patients had a dose reduction to 135 μg/w or 90 μg/w. There was no discontinuation of the NUC regimens in group 1 and only one patient in group 2. At week 48, 7 patients had an HBsAg clearance in group 1 and 1 in group 2 (p = 0.032). Demographic and baseline characteristics, CHB history and history of anti-HBV therapies were studied. HBsAg clearance at the end of PEGIFN treatment (W48) was associated with (1) baseline HBsAg titer (p = 0.020) and (2) history of HBeAg seroconversion prior to randomization [4/19 (21%) vs 3/64 (4.6%), p = 0.038].
Final results regarding primary end point at week 96 will be presented in this meeting.
Conclusions: Addition of a 48 weeks course of PEGIFN alfa-2a to oral anti-HBV therapy in HBeAg negative CHB patients with undetectable serum HBV DNA for at least 1 year: (1) Results in a low rate of HBsAg clearance [7/90 (8%)] and (2) Suggests that low baseline HBs Ag titers and a history of HBeAg seroconversion either spontaneaously or under HBV therapy may increase HBsAg clearance rate.

O113

HLA DPB1 rs9277535 POLYMORPHISM STRONGLY PREDICTS HBsAg CLEARANCE IN IFN TREATED GENOTYPE D HBeAg-NEGATIVE PATIENTS WITH CHRONIC HEPATITIS B

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Background and Aims: HLA DP polymorphisms have recently been associated to spontaneous hepatitis B virus (HBV) clearance in Asians patients. Whether these SNPs influence IFN-induced HBsAg response in difficult to cure HBeAg-negative genotype D chronic hepatitis B Caucasian patients, is currently unknown.
Methods: 126 such patients with compensated disease (46 years, 82% males, 90% genotype D, HBV DNA 6.2 log cp/ml, ALT 132 IU/L, 40% with cirrhosis) were followed for a median of 11 (1–23) years after 22 (6–48) months of either standard or pegylated (Peg)IFN alfa treatment. The primary endpoint was HBsAg clearance. HLA DPA1 rs3077, HLA DPB1 rs9277535 and rs9277534, and IL28B rs12979860 genotypes were assessed by using TaqMan SNP Genotyping Assays.
Results: Twenty-eight (22%) patients ultimately cleared serum HBsAg with a 15-year cumulative probability of 30%, with a preference for patients with baseline lower HBV DNA levels, higher ALT levels, IL28B rs12979860 CC, HLA DPB1 rs9277534 AG/GG and HLA DPB1 rs9277535 AG/GG. At multivariate analysis, HLA DPB1 rs9277535 AG/GG genotype was the strongest independent predictor of HBsAg seroclearance (HR: 6.88; 95% CI 2.82–16–7, p < 0.000) along with baseline ALT levels (HR 1.00, 95% CI 1.00–1.00, p = 0.001), baseline HBV DNA levels (HR 0.54, 95% CI 0.37–0.79, p = 0.002), and IL28B rs12979860 CC genotype (HR 2.2, 95% CI 1.02–5.0, p = 0.044). The 15-year cumulative rates of HBsAg clearance were 62% in carriers of the HLA DPB1 rs9277535 AG/GG genotype compared to 13% in carriers of the AA genotype (p < 0.0000).

Conclusions: HLA DPB1 rs9277535 polymorphism strongly predicts IFN-induced HBsAg clearance in HBeAg-negative patients chronically infected by genotype D HBV. This genetic signature may help to select patients to IFN based regimen.

O114

SERUM HBV-RNA LEVELS DECLINE SIGNIFICANTLY IN CHRONIC HEPATITIS B PATIENTS DOSED WITH THE NUCLEIC-ACID POLYMER REP2139-CA

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Background and Aims: Treatment of chronic hepatitis B (CHB) patients with the HBsAg release inhibitor REP2139-Ca may be a promising new option for achieving therapy-induced HBsAg loss (functional cure), however its effect on circulating hepatitis B pregenomic RNA (HBV-RNA) is not known. For this, we determined HBV-RNA levels during treatment with REP2139-Ca and compared these with HBV-DNA and HBsAg levels.
Methods: 12 Patients with HBeAg positive CHB (mean HBV-DNA 8.21 logC/mL) participating in a phase 2 study were dosed with the nucleic-acid based amphipathic polymer REP2139-Ca for 20–38 weeks. Responders to REP2139 (defined as decline in serum HBsAg) were subsequently treated with add-on peginterferon alpha-2a and/or thymosin alpha-1. HBsAg (Architect), HBV-DNA, and HBV-RNA levels were determined in baseline serum samples, after 20–24 weeks of REP2139-Ca monotherapy, and either during a treatment-free follow-up (for responders) or during entecavir treatment (for non-responders). HBV-RNA isolated from 140 μL of plasma was quantified by RT-qPCR using HBV-specific primers. Lower limit of quantification of RNA was set at 3.00 logC/mL. Variables were evaluated with a paired T-test.
Results: HBV-RNA was detectable in all 12 patients before treatment [mean 6.70 logC/mL (SD 0.83)], and was significantly associated with HBsAg (r^2 0.33, $p = 0.049$) and HBV-DNA (r^2 0.74, $p < 0.001$). After 20–24 weeks of REP2139-Ca treatment, mean HBV-RNA, HBV-DNA, and HBsAg levels declined significantly compared to baseline (−2.54, −3.34, and −3.12 log C or IU/mL, respectively, all $p < 0.001$). At week 20–24, HBV-RNA was undetectable in 8/12 patients. In 7 of these 8 patients, HBV-RNA remained undetectable during the treatment-free follow-up period (mean 21.9 weeks, range 7–27). HBsAg loss and anti-HBs seroconversion was achieved in 4/8 patients during treatment-free follow-up (anti-HBs range 200–766 U/L).
In contrast, 3/4 patients with detectable HBV-RNA at week 20–24 of REP2139-Ca treatment also showed no decline in HBV-DNA or HBsAg, after which entecavir therapy was initiated. After 21–32 weeks of entecavir treatment HBV-DNA had declined significantly (−4.39 logC/mL, $p = 0.029$), whereas HBV-RNA levels remained unchanged (+0.23 logC/mL, $p = 0.188$).
Conclusions: Treatment of CHB patients with REP2139-Ca resulted in a pronounced decline of serum HBV-RNA, HBV-DNA and HBsAg in the majority of patients, and may be a promising new option for improving the chance of functional cure in CHB patients.

O115

HIGH ANTIVIRAL ACTIVITY OF THE HBV CORE INHIBITOR NVR 3-778 IN THE HUMANIZED UPA/SCID MOUSE MODEL

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Background and Aims: NVR 3-778 represents a new class of HBV core inhibitors and is in clinical development for the treatment of chronic hepatitis B. We determined the antiviral activity of NVR 3-778 alone or in combination with PEG-IFN or entecavir in the humanized uPA/SCID mouse model of HBV infection.
Methods: Thirty-six mice infected with HBV genotype C, with serum HBV viremia $>10^6$ copies/ml and human serum albumin levels >6 mg/ml were randomized into 6 treatment groups: (1) NVR 3-778, (2) vehicle, (3) entecavir, (4) PEG-IFN, (5) NVR 3-778 + entecavir and (6) NVR 3-778 + PEG-IFN. Virologic endpoints included serum and intrahepatic HBV DNA, HBV antigen, cccDNA and HBV RNA levels.
Results: All 6 mice in the NVR 3-778 group showed >1.7 log serum viral load reduction from baseline at day 14 [mean (median) viral load reduction 1.9 (1.9) log]. This effect was similar to that obtained with entecavir ($p > 0.05$) and larger than that obtained with PEG-IFN treatment ($p < 0.05$). The largest viremia reduction across all groups was obtained when NVR 3-778 was combined with PEG-IFN, resulting in a mean (median) viral load reduction of 2.4 (2.4) log at day 14, significantly larger than that obtained with either NVR 3-778 or PEG-IFN alone ($p < 0.05$). The combination of NVR 3-778 and PEG-IFN also showed the largest reduction in intrahepatic HBV DNA loads (median 3.0 log; $p < 0.005$) as compared to other treatment groups (NVR 3-778 alone 2.0 log, entecavir 2.2 log and PEG-IFN 1.8 log). Intrahepatic HBV RNA loads were reduced significantly in the PEG-IFN groups only. As expected, serum levels of HBsAg were reduced most strongly in the PEG-IFN groups and were only minimally affected by NVR 3-778 alone. Levels of cccDNA were similar across treatment groups. Treatment with NVR 3-778 was not associated with any significant changes in the levels of human serum albumin, serum alanine aminotransferase (ALT) levels, or intrahepatic amounts of human β-globin, indicating that the number of human hepatocytes remained stable during the treatment period.
Conclusions: The HBV core inhibitor NVR 3-778 demonstrated high intrinsic antiviral activity in HBV infected humanized mice. Serum HBV viral load reduction was larger than that obtained with PEG-IFN and similar to entecavir alone. The combination of NVR 3-778 and PEG-IFN showed higher antiviral activity as compared to NVR 3-778 or PEG-IFN alone, indicating a functionally beneficialinteraction between a core inhibitor and interferon alpha.

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PREDICTIVE VALUE OF BASELINE AND ON-TREATMENT qHBsAg LEVEL IN HBeAg POSITIVE CHB PATIENTS WHO SWITCHED FROM NUCS TO PEGYLATED INTERFERON A-2A: A FURTHER ANALYSIS FROM NEW SWITCH STUDY

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Background and Aims: qHBsAg level, which has been used to predict IFN response in treatment naïve patients, is rarely studied in NUC-treated CHB patients. Aim of this study is to investigate the predictive value of baseline and on-treatment qHBsAg levels on treatment response in patients who switched from a long-term treatment of NUC to pegylated interferon a-2a (PEG-IFN a-2a).
Methods: HBeAg positive CHB patients who achieved partial responses (defined as HBV DNA<200 IU/ml and HBeAg loss) with a prior NUC history for 1–3 years were included in NEW SWITCH study. All participants were switched to PEG-IFN a-2a treatment for either 48 or 96 weeks (with the first 12 weeks overlapping NUC therapy) at a randomization ratio of 1:1 and followed-up to 48 weeks after the discontinuation of PEG-IFN a-2a. The predictive values of qHBsAg levels at baseline, 12wks and 24wks on treatment response (defined as HBsAg loss at week 48) were analyzed in the retrospective analysis.
Results: 271 patients, who had completed 48wks of treatment, were recruited in this analysis, including 47 (17.3%) patients achieved HBsAg loss at week 48. Significantly lower qHBsAg levels at baseline (720.97 IU/ml *vs* 5456.16 IU/ml, $P < 0.001$) and reduction of qHBsAg during treatment ($P < 0.001$) were observed in patients with HBsAg loss comparing with those without.
Patients with qHBsAg<1500 IU/ml at baseline achieved higher HBsAg loss rate than those with qHBsAg≥1500 IU/ml (33.3% *vs* 4.1%, $P < 0.0001$). Similarly, those with qHBsAg levels <200 IU/ml at week 24 achieved higher HBsAg loss rate than those with qHBsAg≥200 IU/ml (48.4% *vs* 0.6%, $P < 0.0001$).
Analysis of the combinative prediction value of baseline and on-treatment qHBsAg levels has shown that patients with qHBsAg<1500 IU/ml at baseline and qHBsAg <200 IU/ml at week 24 had the highest response rate (PPV 51.35%), while those with qHBsAg≥1500 IU/ml at baseline and qHBsAg≥200 IU/ml at week 24 had the lowest response rate (NPV 100%, Figure 1).
Conclusions: Combination of qHBsAg levels at baseline and week 24 might be able to predict HBsAg loss at week 48 in HBeAg positive CHB patients who switched to PEG-IFN a-2a after achieving partial responses in NUC treatment.

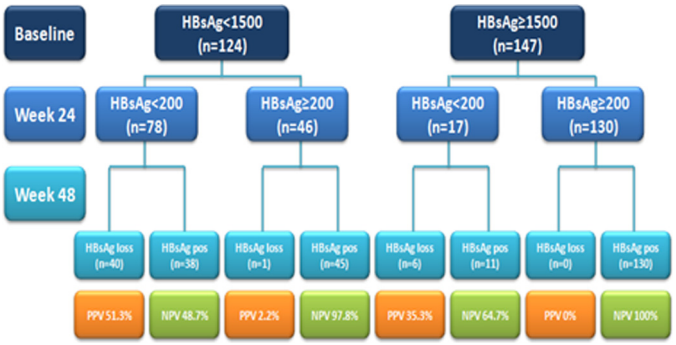

Figure 1. The algorithm for the predictive value of qHBsAg levels.

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PREDICTORS OF CLINICAL RESPONSE: RESULTS FROM A LARGE, RANDOMIZED CONTROLLED STUDY WITH TENOFOVIR DISOPROXIL FUMARATE (TDF) PLUS PEGINTERFERON ALFA-2A (PEG) COMBINATION FOR CHRONIC HEPATITIS B (CHB)

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Background and Aims: There are scarce on-treatment data on HBsAg and HBV DNA kinetics with concomitant nucleos(t)ide

analog and immunomodulator HBV therapy. Predictors of clinical response may help manage patients on combination therapy.
Methods: From study GS-US-174-0149, 740 CHB patients without advanced disease were randomized 1:1:1:1 to receive (TDF+PEG) x48 weeks (arm A); (TDF+PEG) x16 weeks followed by TDFx32 weeks (arm B); continuous TDF (arm C); PEGx48 weeks (arm D). Associations between baseline and on-treatment variables with change in HBV DNA (log_{10} IU/ml) or HBsAg (log_{10} IU/ml) levels from baseline to week 48 or HBsAg loss at Week 72 were examined via linear regression or Cox regression analyses, respectively, in univariate models as well as multivariate models.
Results: In a multivariate analysis, treatment arms A, B, and C compared to arm D, baseline HBeAg-negative status, higher baseline HBV DNA, and lower baseline HBsAg levels were associated with greater HBV DNA decline at Week 48. For every 1 log_{10} IU/ml increase in baseline HBV DNA or 1 log_{10} IU/ml decrease in baseline HBsAg, there is an expected greater HBV DNA decline by 0.85 or 0.31 log10 IU/mL, respectively, at Week 48. On multivariate analysis, achieving HBsAg loss was associated with treatment Arm A, GT A, early ALT flare on treatment, and, at Week 12, with HBsAg decline from baseline >1 log_{10} IU/ml (table). The positive predictive values for achieving HBsAg loss at week 72 with (TDF+PEG) $\times$48 weeks with either 1 log_{10} IU/ml decline or HBsAg <100 IU/ml at week 12 were 43% and 50%, respectively, and the negative predictive values (NPV) were 97% and 95%, respectively. TDF+ PEG combination for 48 weeks exhibited the largest HBsAg decline (log_{10} IU/ml) as compared to other treatment arms (Arm A: −1.1, B: −0.5, C: −0.3, D: −0.8; $p < 0.01$ for all comparisons of arm A versus others). Similar levels of HBsAg decline were observed in genotypes (GT) A and B (mean log_{10} IU/ml $\pm$ SD: −1.2$\pm$2.0 and −1.1$\pm$1.2, respectively), both of which exhibited a greater decline than GT C and D (−0.5$\pm$0.9 and −0.4$\pm$1.0, respectively) ($p < 0.05$).

Table: Predictors of HBsAg loss at Week 72 – Multivariate Cox Regression Analysis (Number of observations: 625)

Predictors	Hazard ratio	95% CI	p-value
Arm A vs. B	3.68	(1.25, 13.32)	0.033
Arm A vs. D	3.82	(1.29, 13.97)	0.032
GT A vs. B	8.90	(2.63, 32.22)	<0.001
GT A vs. C	15.51	(3.95, 73.96)	<0.001
GT A vs. D	7.88	(1.84, 46.78)	0.015
Achieving >1 log_{10} IU/ml decline in HBsAg at week 12: Y vs. N	7.80	(2.05, 29.22)	0.003
ALT flare in 1st 12 weeks: Y vs. N	4.65	(1.54, 14.46)	0.009

Conclusions: Higher baseline HBsAg levels appear to impact negatively on-treatment HBV DNA response. HBsAg decline on TDF plus PEG combination therapy x48weeks was synergistically greater than on either TDF or PEG monotherapy. HBsAg decline appears to favor HBV GT A and B. Moreover, HBsAg decline at week 12 shows high NPV for week 72 HBsAg loss and may provide a valuable tool for response-guided therapy in PEG+TDF combination treatment.

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OPTIMIZING THE PRENYLATION INHIBITOR LONAFARNIB USING RITONAVIR BOOSTING IN PATIENTS WITH CHRONIC DELTA HEPATITIS

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Background and Aims: Chronic delta hepatitis (CDH) is the most severe form of chronic viral hepatitis. Interferons are effective in only a quarter of patients and alternative treatment options are urgently needed. Prenylation inhibitors (PI) function by impeding virion assembly. In a proof of concept study, 100 mg BID or 200 mg BID of the PI lonafarnib (LNF) led to a 0.7 log and 1.6 log reduction in serum HDV RNA, respectively, after 4 weeks of treatment (Koh et al, AASLD 2014). AIM: To explore and identify an optimal LNF-based treatment regimen for further development.
Methods: To date, a total of 15 patients have received LNF alone, or in combination (**LO**nafarnib **W**ith **R**itonavir for **HDV** – **LOWR HDV-1**). 9 patients received LNF as monotherapy (LNF 200 mg BID, n = 3; LNF 300 mg BID, n = 3; LNF 100 mg TID, n = 3). 3 patients received LNF 100 mg BID in combination with 180 ug pegylated interferon alfa 2a QW, and 3 patients in combination with ritonavir 100 mg QD. Treatment durations ranged from 4 to 12 weeks. All patients underwent 72-hour pharmacokinetic and pharmacodynamic evaluations Biochemical parameters, quantitative HDV RNA and HBV DNA were tested at days 1, 2, 3, 7, 14, 28 and at 2–4 week intervals thereafter, as well as post-treatment.
Results: LNF + RTN produced the most optimal regimen with HDV RNA decline, ALT normalization and and acceptable safety and tolerability. LNF + RTN combination achieved the best HDV RNA decline after 4 weeks (mean reduction: 2.21 log_{10} copies/mL $\pm$ 0.53 [x$\pm$SD], $p < 0.001$ vs baseline). After 8 weeks of LNF + RTN combination, mean reduction of HDV RNA was 3.45 log, with 1 of 3 patients becoming HDV RNA negative. In other treatment groups, mean HDV RNA decline after 4 weeks ranged between 1.18 and 1.52 log_{10} copies/mL. Adverse events during treatment included anorexia, nausea, diarrhea and weight loss of grade 1 and 2 (according to CTCAE criteria). Mean serum LNF concentrations increased by more than 3 times with RTN boosting compared to LNF monotherapy at all time points tested. RTN-boosted LNF 100 mg BID not only achieved the greatest reductions in HDV RNA, but this regimen was better tolerated than higher doses of LNF.
Conclusions: 1. LNF is effective in human CDH. 2. Based on the results to date, it was decided to further develop LNF with RTN boosting and 12 additional HDV infected patients are currently receiving LNF + RTN (**LOWR HDV-2**). The data from **LOWR HDV-1** and the ongoing **LOWR HDV-2** trials will be presented at the EASL meeting.

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STOPPING TENOFOVIR DISOPROXIL FUMARATE (TDF) TREATMENT AFTER LONG TERM VIROLOGIC SUPPRESSION IN HBeAg-NEGATIVE CHB: WEEK 48 INTERIM RESULTS FROM AN ONGOING RANDOMIZED, CONTROLLED TRIAL ("FINITE CHB")

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Background and Aims: Long-term effective NUC therapy may lead to partial restoration of HBV-specific T cell functions. Stopping therapy in HBV-DNA suppressed HBeAg-negative patients may lead to initial viral rebound and hepatic flare followed by HBsAg clearance. We investigated HBsAg kinetics after controlled stopping of long-term TDF therapy.
Methods: Subjects on effective TDF therapy for at least 4 years were randomized to either stop or to continue TDF therapy for 144 weeks (advanced fibrosis/ cirrhosis excluded). Primary endpoint is HBsAg loss at Week 144. TDF could be restarted in case of clinically significant hepatitis B flares.
Results: 45 subjects were randomized in this open-label study at 13 sites in Germany (median age 45 years, 79% male, 88% Caucasian). 21 Stop-TDF subjects and 21 Continue-TDF subjects completed Week 48 (3 subjects withdrew consent – data excluded). At W48, Continue-TDF subjects maintained viral suppression, stable ALT, none lost HBsAg. 19 of 21 Stop-TDF subjects had early (first 12 weeks) substantial HBV DNA rebound (median 205,380 IU/mL, [Q1 59,995 IU/mL; Q3 444,147 IU/mL]) accompanied by ALT elevations (median 106 IU/mL; [Q1 76 IU/ml; Q3 233 IU/mL]). 2 subjects had minimal HBV DNA rebound (max 259 IU/mL) and normal ALT; both had HBsAg levels <500 IU/ml at baseline (BL), while HBsAg levels in the other 19 subjects were substantially higher (median 52,640 IU/mL; [Q1 26,518 IU/mL; Q3 95,240 IU/mL; range 4,400–221,840 IU/mL]). At W48, 3 subjects had restarted TDF and all had returned to normal ALT and HBV DNA <LLQ, 18 subjects remained off TDF, 15/18 had normal ALT, 14/18 had HBV DNA <2000 IU/mL, and 2/18 lost HBsAg (both male, ages 46 and 58 years, BL HBsAg 7,308 and 15,276 IU/mL, respectively). Among subjects that did not restart TDF, BL HBsAg levels were lower in those with HBsAg decline >1 log (n = 5, median −1.62 log) compared those with decline <1 log (n = 13, median −0.23 log); median BL HBsAg levels were 14,888 IU/ml vs 52,280 IU/ml, respectively. Continue-TDF subjects had only small declines in HBsAg (median: −0.09 log).
Conclusions: Stopping TDF in chronic HBV HBeAg negative long-term suppressed subjects with defined restarting criteria appears to be safe and led to a significantly greater early HBsAg decline as compared to a continued TDF monotherapy. HBsAg loss was observed so far in two subjects (9.5%). If necessary, TDF can be effectively restarted. Lower HBsAg level at BL seems to be a predictive factor for HBsAg decline.

EU and Public Health

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ADVANCED FIBROSIS IS COMMON IN INDIVIDUALS WHOSE HEPATITIS C HAS NOT BEEN DIAGNOSED: RESULTS FROM THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 2001–2012

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Background and Aims: Hepatitis C virus (HCV) infection is a global public health problem – while it is common, its consequences may be severe, including end stage liver disease and hepatocellular carcinoma. Moreover, most individuals with HCV remain asymptomatic, which makes the diagnosis difficult. With the hypothesis that individuals whose HCV is not diagnosed are less likely to have advanced fibrosis than those who have been diagnosed, we compare liver fibrosis between respondents to the National Health and Nutrition Examination Survey (NHANES) with diagnosed and undiagnosed HCV infection.
Methods: Testing for HCV was incorporated in NHANES 2001–2012. In a subgroup of the respondents with HCV infection, follow-up questionnaires were administered. Awareness of HCV infection was assessed by the question whether they had known they had HCV before receiving a letter from NHANES. Liver fibrosis was estimated by the FIB-4 and APRI scores. Based on the published cut-off values for advanced fibrosis, the proportion of respondents with a high probability of advanced fibrosis was compared between respondents with known and undiagnosed HCV.
Results: Out of 30,140 respondents of the NHANES survey, 360 tested positive for HCV RNA. There were 355 participants with complete laboratory data needed for the FIB-4 and APRI scores, of whom 130 had completed the full hepatitis C follow-up questionnaires. Slightly less than half (47.7%, n = 62) knew that they had hepatitis C infection before the survey, whereas in the remainder (52.3%), HCV was only discovered from the survey. In the figure, the two groups were comparable with respect to age, sex, aminotransferase and platelet counts. BMI was higher in those with known diagnosed, the significance of which is uncertain. The raw FIB-4 and APRI scores were similar between the two groups. Among the respondents with known HCV infection, the proportion with a high, intermediate, and low probability of advanced fibrosis was 14.5%, 40.3%, 45.2%, respectively. The corresponding data in those with undiagnosed HCV were 19.1%, 30.9%, 50.0%, respectively. A similar pattern was seen with the APRI score.

	Aware	Not aware	p
N	62	68	0.59
Age	52.1	51.2	0.64
Male %	66.7%	76.5%	0.21
AST	57.1	58.0	0.89
ALT	58.1	61.7	0.62
Platelets	216	236	0.18
BMI	30.3	27.1	<0.01
FIB-4 score	2.32	2.14	0.62
	14.5% Advanced	19.1% Advanced	0.53
APRI score	0.90	0.82	0.63
	17.7% Advanced	17.7% Advanced	0.21

Conclusions: While more than half of survey respondents did not know of their HCV infection, their liver fibrosis was no less advanced than those whose HCV had been diagnosed prior to

participation in the survey. These data further justifies the current recommendation for HCV screening in asymptomatic individuals.

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THE USE OF A POCKET-SIZED ULTRASOUND DEVICE IMPROVES PHYSICAL EXAMINATION: RESULTS OF AN IN- AND OUTPATIENT STUDY

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Background and Aims: The performance of pocket-sized ultrasound devices (PUDs) is comparable with that of standard ultrasonography, whereas the accuracy of a physical examination is often poor requiring further tests to assess diagnostic hypotheses. Adding the use of PUD to physical examination could lead to an incremental benefit. Accordingly, present cohort impact study was aimed at assessing whether the use of PUD in the context of physical examination could reduce the request rate of additional tests when used by physicians in different clinical settings.
Methods: The study involved medical wards (#4), hepatological outpatient clinic (#1) and 90 GPs operating in the same geographical area. After a short predefined training course 135 physicians used PUD in addition to physical examination in 1962 consecutive patients to investigate ten well-defined clinical hypotheses (biliary-duct dilation/ gallstones, ascites, splenomegaly, pleural /pericardial effusion, urinary retention/ stones, abdominal mass/ aortic aneurysm). PUD related findings were recorded. and the decision as to whether to request further tests was left to the physician clinical judgement. The main outcome was to define the proportion of cases undergoing additional tests after PUD. An accurate report of the frequency of the clinical questions in the different settings was also planning, using logistic regression analysis to assess the determinant(s) of the primary outcome.
Results: Of the 1962 patients included 726 (37%) were inpatients, 510 (26%) hepatology outpatients and 726 (37%) recruited from GPs. Gallstones (37%), ascites (17%), pleural effusion (13%), urinary stones (13%) and urinary retention (12%) accounted for >90% of the clinical questions, confirmed by PUD in 66% of cases. The overall frequency of further tests after PUD was 37%; and logistic was associated with both the clinical questions and settings ($p < 0.01$). The rate of agreement between findings at PUD and additional tests was 89%, with a sensitivity of 91% and a specificity of 83% (LR+ 5.4; LR− 0.11).
Conclusions: After a simple and short training, a PUD examination can be used in addition to a physical examination in both in- and outpatients to improve the answer to ten common clinical questions, thus reducing the need further testing.

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CLINICAL IMPACT OF FIVE LARGE-SCALE SCREENING PROJECTS FOR CHRONIC HEPATITIS B AND C IN CHINESE MIGRANTS IN THE NETHERLANDS

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Background and Aims: Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection represent a global public health problem resulting in significant morbidity and mortality. In high endemic regions primary prevention in the form of HBV vaccination has led to a decrease in morbidity and mortality. In low endemic countries it is debated whether it is useful to screen for chronic HBV and HCV in first generation migrants from high endemic countries. In the current report, we describe the outcome in terms of clinical impact of five large-scale Dutch screening projects in which screening for HBV and HCV was offered to first generation Chinese migrants aiming at secondary prevention.
Methods: Between 2009 and 2013 five outreach screening projects for viral hepatitis targeting first generation Chinese migrants were conducted in five main Dutch cities. In three cities all HBsAg positive patients were referred for further evaluation to a medical specialist, in two cities only when either HBeAg was positive or ALT was elevated. To explore the relevance of our screening projects we defined clinical impact as the presence of an indication for: 1. start of antiviral therapy within one year of follow-up 2. strict follow-up because of high HBV DNA levels (HBV DNA >20,000 IU/ml) and/or 3. surveillance for HCC according to the AASLD and Dutch guidelines.
Results: In total, 4423 persons participated in the screening projects of which 6% (n = 264) was HBsAg positive. The total number of newly diagnosed HBsAg positive patients who were analysed in specialist care was 129. Among these patients prevalence of cirrhosis was 7%. Within one year of initial consultation, antiviral therapy for HBV was started in 32 patients (25%). In patients without an initial treatment indication, strict follow-up because of high HBV DNA levels and/ or HCC surveillance was considered indicated in 64 patients (50%). Three patients (0.7%) were newly diagnosed with chronic HCV infection (two of these with HBV co-infection). Overall, 75% of all HBsAg and HCV positive patients needed antiviral treatment within one year of follow up or were thought to need strict follow up or HCC surveillance.
Conclusions: In our screening project in first generation Chinese migrants, in 75% of all positive HBsAg and HCV patients analysed

in referral centres, antiviral treatment, strict follow-up because of high HBV DNA levels and/ or HCC surveillance were considered indicated. These data show that detection of HBV infection had considerable impact on patient care.

O123

TEN YEARS OF HOSPITAL ADMISSIONS FOR LIVER CIRRHOSIS IN PORTUGAL

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Background and Aims: Robust data on epidemiology of liver diseases in Europe are lacking. Furthermore, recent changes in alcohol and drug consumption may be affecting the burden of liver cirrhosis (LC). We aimed at characterizing hospital admissions for LC in Portugal in the past decade.

Methods: We analyzed all hospital admissions for LC at Portugal Mainland from patients aged ≥20 years between 2003 and 2012 registered in the Diagnosis-Related Group database from the Portuguese Health System's Central Administration. Selected admissions had LC as main diagnosis; or a complication of LC as main diagnosis with a concomitant secondary diagnosis suggestive of LC. Cirrhosis was classified according to etiology considering alcohol, hepatitis B and hepatitis C.

Results: Between 2003 and 2012 there were 63,910 admissions for LC in public institutions in Portugal Mainland, 74.4% were from male patients. Causes of admitted cirrhosis were: 76.0% alcoholic, 1.1% hepatitis B, 1.4% hepatitis B plus alcohol, 3.6% hepatitis C, 4.0% hepatitis C plus alcohol. There was a significant decline ($p < 0.001$) in admissions for alcoholic cirrhosis, whereas hospitalizations for cirrhosis due to hepatitis C or hepatitis C plus alcohol raised by 50% ($p < 0.001$). Admitted patients with alcoholic plus hepatitis B or C cirrhosis were significantly younger than those with either alcoholic or viral cirrhosis (53.1 years vs 59.4 years respectively, $p < 0.001$). Hospitalization rates for LC in 2011 were 124.4/100,000 in men and 32.6/100,000 in women. Hepatocellular carcinoma and fluid retention were more common in viral cirrhosis, while encephalopathy and variceal bleeding were more frequent in alcoholic cirrhosis. Hepatorenal syndrome was the strongest predictor of in-hospital mortality (OR 13.09; 95% CI 12.07–14.22) compared to spontaneous bacterial peritonitis (OR 2.05; 95% CI 1.86–2.26), hepatocellular carcinoma (OR 1.92; 95% CI 1.82–2.03), hepatic encephalopathy (OR 1.92; 95% CI 1.85–1.99), hepatic hydrothorax (OR 1.52; 95% CI 1.41–1.64), esophageal variceal bleeding (OR 1.34; 95% CI 1.27–1.42), age ≥50 years (OR 1.20; 95% CI 1.14–1.26), ascites (OR 1.00; 95% CI 0.93–1.06) and patient gender (OR 1.01; 95% CI 0.96–1.05). In-hospital mortality was 15.2% in LC from all causes.

Conclusions: Despite the decline in admissions for alcoholic cirrhosis and the rise of those related to hepatitis C, the burden of hospitalized liver cirrhosis in Portugal was essentially attributable to alcoholic liver disease.

O124

IS INCREASED HCV CASE-FINDING COMBINED WITH 8 OR 12 WEEK INTERFERON-FREE DIRECT-ACTING ANTIVIRAL TREATMENT COST-EFFECTIVE IN UK PRISONS? A COST UTILITY ANALYSIS INCLUDING TREATMENT AS PREVENTION BENEFITS

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Background and Aims: Hepatitis C virus (HCV) prevalence is high among incarcerated populations, and in 2014 England introduced opt-out HCV testing in prisons. However, short sentences for people who inject drugs (PWID) in the UK combined with long HCV treatments meant case finding was unlikely to be cost-effective without considerable continuity of care between prison and the community, which could not be guaranteed. We assess the cost-effectiveness of increased testing and treatment in UK prisons using 8–12 week interferon-free direct-acting antiviral (DAA) HCV regimens including prevention benefits.

Methods: A previously developed dynamic model of incarceration and HCV transmission was used to assess the cost-effectiveness of doubling HCV case-finding in UK prisons (achieved in one site) when combined with 12 or 8 week IFN-free DAAs including individual and population prevention benefits. The intervention is compared to a baseline of current prison testing and IFN/RBV treatment. Costs (in GBP £) and health utilities (in quality-adjusted life-years, QALYs) were used to calculate mean incremental cost effectiveness ratios (ICERs). We assume 95% SVR and a cost of £3200/wk for DAAs. Based on UK prison data, we model a baseline testing rate of 1.5% per month, 15% yield, and a 56% referral rate. We assume 25%/2.5% exPWID/PWID who attend referral, respectively, are initiated onto treatment within 2 months of diagnosis, PWID and ex/nonPWID are in prison an average 4 and 8.5 months, respectively, We assume no continuity of treatment between prison and community. Multivariate probabilistic sensitivity analyses were performed.

Results: Doubling prison testing rates and treatment with 12 week DAAs produces a mean ICER of £25,766 per QALY gained compared to current testing and treatment, and is 65% likely to be cost-effective under a £30,000 willingness to pay threshold (Figure 1a). Doubled prison treatment rates (achieved in the community) lowers the ICER to £21,678/QALY due to additional individual and population prevention benefits. With 8 week DAAs, the ICER drops to £18,715/QALY and is 88% likely to be cost-effective under a £30,000 threshold (Figure 1b).

Conclusions: Increased HCV case-finding and treatment using short duration DAAs is likely to be cost-effective in UK prisons due to

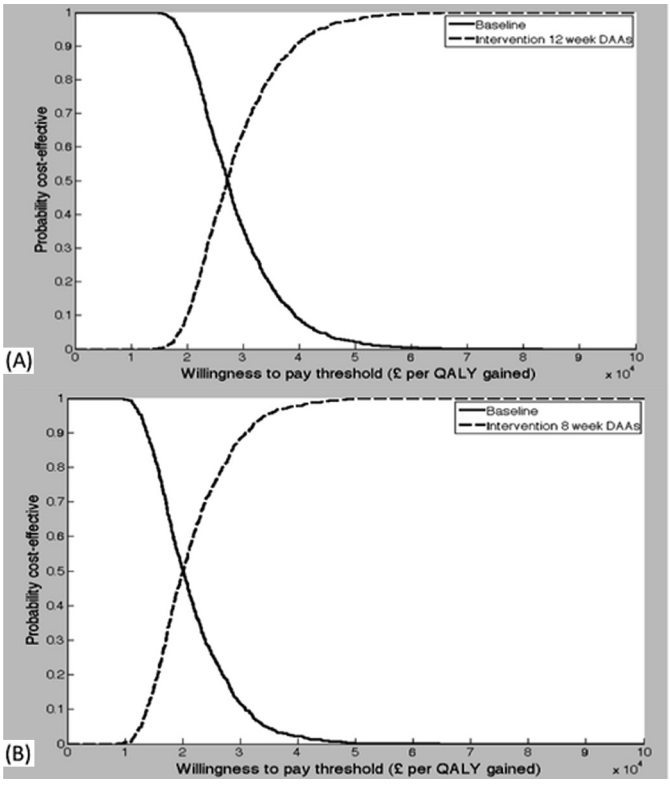

Figure 1. Cost-effectiveness acceptability curves for doubled HCV case-finding in prison combined with (A) 12- and (B) 8-week IFN-free DAAs.

higher completion rates. Greater cost-effectiveness can be achieved through scale-up of treatment due to treatment as prevention benefit, but the affordability of scaling up treatment sufficiently to reduce community transmission is unclear.

O125
A SYSTEMATIC REVIEW OF HEPATITIS B AND C TESTING IN THE COUNTRIES OF THE WHO EUROPEAN REGION

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Background and Aims: Growing awareness of the threat posed by hepatitis B and C has been accompanied by important biomedical advances in their treatment. However, in Europe as elsewhere, there is the potential for hepatitis drugs to be greatly underutilised because many people who might benefit from them are undiagnosed. We reviewed scientific studies reporting hepatitis B and C testing as a step toward informing public health strategies to reduce the number of individuals who remain undiagnosed.
Methods: Using PRISMA criteria, we conducted a systematic review of the MEDLINE and EMBASE databases to identify original research studies reporting levels of hepatitis B and C testing in the 53 Member States of the WHO European Region. English-language peer-reviewed articles and conference abstracts published between January 2007 and June 2013 were included.
Results: This review identified 154 studies from 28 (52.8%) of 53 countries. More than two-thirds of the studies (67.7%) were from six countries: Turkey, Germany, Italy, France, the Netherlands and the United Kingdom. The populations studied most frequently were people who use drugs (32 studies), health care patients (28) and populations tested for reasons relating to pregnancy or use of assisted reproductive technology (18) (Figure 1). Median testing uptake levels ranged from 100% for eight types of populations (Figure 1) to 79.9% for people born to HBsAg-positive mothers and 70.5% for current or former prison inmates. Four studies reported testing uptake of 75.0% or lower in people living with HIV, and six studies reported testing uptake of less than 50% in people who use drugs. The highest median HBsAg prevalence (14.9%) and HCV RNA prevalence (49.7%) were both found in people who use drugs.

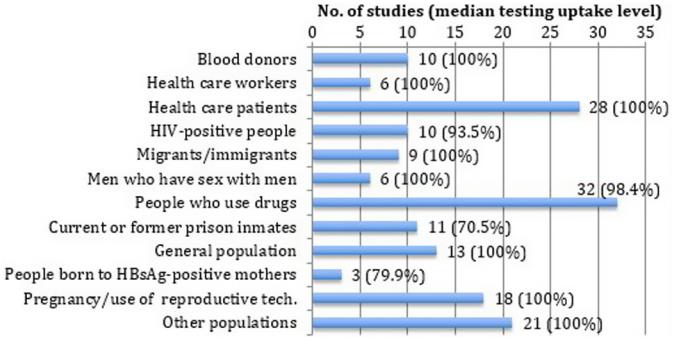

Figure 1. Number of studies reporting on each type of population. [Numbers sum to more than the total number of studies in the review because some studies reported on more than one type of population.]

Conclusions: An evidence base on hepatitis B and C testing appears to be lacking in many European countries. The results indicate that some high-risk populations have been studied much more than others, but mostly only in a small number of countries. Since almost all studies utilised methodologies that required or encouraged study participants to undergo testing, high median testing uptake levels are not likely to be representative of overall testing uptake in most populations. Low testing uptake in some studies raises the question of whether key opportunities to identify infected individuals are being missed. Public health officials need much more comprehensive information in order to plan effective responses to hepatitis B and hepatitis C in Europe.

O126
IMPACT OF SUCCESSIVE HBV-VACCINATION PUBLIC POLICIES ON THE VACCINATION COVERAGE AND INCIDENCE OF HBV INFECTION IN A LARGE FRENCH COHORT OF INDIVIDUALS BORN BETWEEN 1960 AND 1994

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Background and Aims: Hepatitis B remains a pandemic with 240 million chronically infected persons at risk of developing cirrhosis or hepatocellular carcinoma despite the existence of efficient and safe vaccines. To control HBV transmission, the WHO recommends universal vaccination rather than targeting only high risk groups as preferred by several European countries. The French health authorities targeted these risk groups from 1982 to 1994, switched to universal vaccination of infants and catch-up immunization of the general population, to finally abandon catch-up vaccination in 1998. The study aimed at assessing the consequences of these successive vaccination policies on vaccination coverage (VC) and virus circulation among populations at risk of contracting HBV.
Methods: HBV status of 57,113 individuals attending HIV and hepatitis infection screening facilities in Grand Lyon, France, was established by serum HBsAg, anti-HBs, and anti-HBc determination between 2005 and 2010. VC and HBV incidence were calculated for each birth cohort from 1960 to 1995 using statistical "survival" models.
Results: VC increased during the first period corresponding to birth cohorts from 1960 to 1985, reaching a maximum of 60–70% vaccinated individuals in 1983–1985 cohorts. VC then decreased dramatically in post-1985 birth cohorts and fell below 30% in the most recent cohort studied. The incidence of HBV infection in non-vaccinated individuals fluctuated between 5 and 7‰ in pre-1986 birth cohorts, decreased to 2.5‰ in 1986–1991 cohorts but rebounded up to 5‰ in post-1991 birth cohorts.
Conclusions: Reaching a VC above 50% reduced strongly the incidence of HBV infection but the modification of HBV vaccination policies following public controversies in France was sanctioned by a dramatic decline in VC of young adults and a sharp rebound of HBV circulation. These data support the idea that efforts combining universal and catch-up campaigns are essential to control HBV circulation in low endemic countries with the persisting risk to virus resurgence if VC shrinks.

O127
A WORLDWIDE STUDY REVEALS THAT THE AMOUNT OF DAILY ALCOHOL INTAKE IS A BETTER PREDICTOR OF THE WEIGHT OF ALCOHOL IN THE CIRRHOSIS BURDEN THAN THE TOTAL PER CAPITA COMSUMPTION

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Background and Aims: Approximately 6% of global deaths are caused by alcohol consumption, with a major contribution from

alcoholic cirrhosis (Global Status Report on Alcohol and Health, WHO, 2014). Moreover, of diseases not explicitly defined by alcohol consumption, cirrhosis has the highest alcohol-attributable fraction (50% of all cases worldwide). Most studies assessing the prevalence of alcohol abuse as a risk factor for alcoholic cirrhosis focus on total annual per capital consumption. However, clinical studies indicate that high daily consumption is the strongest predictor of alcoholic cirrhosis. We hypothesize that the counties with the highest daily alcohol consumption are at highest risk of having a greater burden of alcoholic cirrhosis.

Methods: We performed a comprehensive analysis of the WHO Global Status Report on Alcohol and Health, which included parameters of alcohol consumption and drinking patterns from 193 countries. We categorized countries by heavy or moderate drinking using the average daily consumption among drinkers, according to the US Dietary Guidelines threshold for heavy drinking (up to 1 drink/day for women, up to 2 drinks/day for men). Moreover, global rates of HCV and HBV were obtained from published seroprevalence estimates, and obesity data were obtained from the WHO Data Repository. Uni- and multivariate models were fitted to determine the correlation between alcohol parameters and the outcome measure of alcohol-attributable fraction (AAF) of cirrhosis, adjusting for the cofactors' effects.

Results: In an integrative model that adjusted for cirrhosis cofactors, the classification of countries by moderate or heavy daily drinking had the strongest influence on the weight of alcohol in the cirrhosis burden. Of note, the cirrhosis burden due to alcohol increased by 11.13% with a transition from the moderate to heavy classification ($p < 0.001$). In contrast, total yearly per capita consumption had a correlation coefficient of only 2.22 with the AAF of cirrhosis ($p < 0.001$). Importantly, drinking patterns such as heavy episodic drinking and the type of alcoholic beverages (wine, beer or spirits) did not independently correlate with AAF.

Conclusions: The presence of heavy daily drinkers in a population most significantly and independently influences the weight of alcohol in a country's cirrhosis burden. Reducing heavy drinking should therefore be considered as an important target for public health monitoring and policies.

Late breaking abstracts – Orals

LO1

SAFETY OF OMBITASVIR/PARITAPREVIR/RITONAVIR PLUS DASABUVIR FOR TREATING HCV GT1 INFECTION IN PATIENTS WITH SEVERE RENAL IMPAIRMENT OR END-STAGE RENAL DISEASE: THE RUBY-I STUDY

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Introduction: Limited data are available on the safety of direct-acting antivirals (DAAs) for treating hepatitis C virus (HCV) infection in patients with severe renal impairment or end-stage renal disease; many go untreated and are vulnerable to disease progression. RUBY-I is an ongoing, open-label study evaluating the interferon-free 3 DAA (3D) combination of ombitasvir/paritaprevir*/ritonavir (25/150/100 mg QD) and dasabuvir (250 mg BID) with or without ribavirin (RBV, 200 mg QD) in patients with stage 4 or 5 chronic kidney disease (CKD) and HCV genotype (GT) 1 infection. We report preliminary safety results from Cohort 1 of this study.

Material and Methods: Treatment-naïve, non-cirrhotic adults with HCV GT1 infection and CKD classified using estimated glomerular filtration rate (eGFR) as stage 4 (eGFR 15–30 mL/min/1.73m^2) or stage 5 (eGFR <15 mL/min/1.73m^2 or requiring dialysis) receive 12 weeks of treatment with 3D with RBV (GT1a) or without RBV (GT1b), with 24 weeks post-treatment follow-up. On-treatment adverse events (AEs), serious AEs and notable laboratory abnormalities through 18 Feb 2015 are reported.

Table: Baseline characteristics

Variable	3D±RBV (N = 17)
Age, yr; mean (SD)	59.5 (5)
Male; n (%)	14 (82)
Race; n (%)	
Black	11 (65)
White	6 (35)
Hispanic or Latino Ethnicity; n (%)	3 (18)
Degree of fibrosis: n (%)	
F0–F1	9 (53)
F2	5 (29)
F3	5 (18)
HCV viral load, $\log_{10}$(IV/ML); median (minimum, maximum)	6.57 (5.47, 7.64)
GT1a; n (%)	12 (71)
CKD stage; n (%)	
4 (eGFR 15–30 mL/min/1.73m^2)	7 (41)
5 (eGFR <15 mL/min/1.73m^2 or requiring dialysis)	10 (59)
On dialysis; n (%)	11 (65)
eGFR, ml/min/1.73m^2; median (minimum, maximum)	10.6 (5.4, 29.9)
Creatinine, mg/dL; median (minimum, maximum)	6.5 (2.2, 10.8)

Results: 17 (of 20 planned) patients in Cohort 1 received study drug as of 18 Feb 2015; six have completed treatment. 13 of 17 patients reported at least 1 AE, mainly mild or moderate in severity. Two patients experienced serious AEs considered unrelated to DAAs (1 patient: diskitis, respiratory failure. 1 patient: pseudoaneurysm, hemoperitoneum, small bowel obstruction). Both briefly interrupted 3D due to complications of hospitalization; no other patient had an interruption or discontinuation of 3D. Six patients met hemoglobin criteria for interruption of RBV (hemoglobin decrease >2 g/dL during any 4-wk period, or a value <10 g/dL at any time). There was one case of hemoglobin <8 g/dL (hospitalized patient with diskitis). No blood transfusions were performed. All viral loads suppressed rapidly on treatment and there are no cases of virologic rebound or relapse to date.

Conclusions: Among HCV GT1-infected patients with stage 4 or 5 CKD in this ongoing trial, the regimen of 3D±RBV has been well tolerated to date, with no treatment-related serious AEs, one hemoglobin decline to <8 g/dL, and no premature discontinuations of DAAs. All viral loads suppressed rapidly on treatment and there are no virologic failures to date. Available pharmacokinetic and SVR data will be presented.

*Paritaprevir was identified by AbbVie and Enanta.

LO2

SIGNIFICANT REDUCTION OF HBsAg AND HDV RNA BY THE NUCLEIC ACID POLYMER REP 2139 IN CAUCASIAN PATIENTS WITH CHRONIC HBV/HDV CO-INFECTION

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Introduction: Nucleic acid polymers (NAPs) block the assembly of HBV subviral particles (SVP), thereby inhibiting their release from infected hepatocytes and eliminating the HBsAg protein from the

blood. The NAP REP 2139 has previously been shown to efficiently clear HBsAg from the blood of patients with HBV mono-infection and improve the ability of immunotherapy to elicit SVR in Asian patients.

Material and Methods: In a phase II proof of concept trial (NCT02233075), the safety and antiviral efficacy of REP 2139 (first in monotherapy and then with add on PEG IFN at week 16) in 12 Caucasian patients with chronic HBV / HDV co-infection is being assessed. Recruitment in this trial is now complete and preliminary safety and efficacy data from for the first 7 patients to reach 13 weeks of REP 2139-Ca monotherapy exposure are disclosed.

Patients with chronic HBV / HDV co-infection are being treated once weekly with 500 mg REP 2139-Ca (calcium chelate complex) by 2h IV infusion. Viremia (HDV RNA and HBV DNA), HBsAg and anti-HBs are followed every two weeks using standard assays (Robogene RT-PCR, Abbott RealTime HBV, Abbott Architect) and performed at the Institute of Virology, University of Duisburg-Essen, Germany.

Results: REP 2139-Ca treatment in all patients is currently well tolerated. All patients have experienced reductions in serum HBsAg and HDV RNA on treatment as outlined in the table below. Four patients have achieved HBsAg reductions of 4–5 logs from baseline with accompanying 5–8 log reductions in HDV RNA (currently undetectable). More moderate HBsAg reductions (1–2 logs) in the other three patients were accompanied by similarly moderate (1–3 log) reductions in HDV RNA. Significant elevations in serum free anti-HBs are detectable in 6/7 patients and are >10 mIU/ml in 4 patients. In the 5 patients with pre-treatment HBV DNA <10 IU/ml, small amounts of detectable HBV DNA are now present.

Patient	HBsAg (IU/ml)		HDV RNA (U/ml)*		Anti-HBs (mIU/ml)		HBV DNA (IU/ml)**	
	Baseline	Week 13	Baseline	Week 13	Baseline	Week 13	Baseline	Week 13
1	13,988	1.17	394,000	TND	1.0	7.9	<10	33
2	27,264	0.34	47,100,000	TND	1.2	25	<10	20
3	28,261	0.01	697,000	TND	0	20.6	<10	25
4	17,511	130	5,490,000	21,500	2.1	6.0	726	66
5	16,426	2,335	211,000	21,100	0	11	104	55
6	12,382	0.07	12,100,000	TND	0.5	26	<10	29
7	20,869	1,732	23,000,000	75,500	0.2	1.3	<10	50

*LLOQ = 1800 U/ml, **LLOQ = 10 IU/ml; TND = RT-PCR target not detected.

Conclusions: REP 2139-Ca is able to achieve rapid reductions in serum HBsAg in Caucasian patients with HBV / HDV infection, demonstrating the reliability of the NAP pharmacological effect (HBsAg reduction or complete clearance) in Caucasian patients. HDV RNA reductions were correlated with HBsAg reductions, suggesting a link between SVP formation and HDV formation. REP 2139-Ca may become an important new therapeutic option for patients with chronic HBV / HDV infection.

LO3

SAFETY AND EFFICACY OF THE COMBINATION DACLATASVIR–SOFOSBUVIR IN HCV GENOTYPE 1-MONO-INFECTED PATIENTS FROM THE FRENCH OBSERVATIONAL COHORT ANRS CO22 HEPATHER*

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Introduction: Real-life results of the Sofosbuvir/Simeprevir combination have been extensively reported but there are few data regarding the Sofosbuvir/Daclatasvir combination. In January 2015, 3003 patients of the French observational cohort ANRS CO22

HEPATHER were given the new oral antivirals in 32 centers: we report the preliminary results of the Sofosbuvir/Daclatasvir combination in Genotype 1-infected patients.

Material and Methods: Demographics, history of liver disease were collected at entry in the cohort. Clinical, adverse events, and virological data were collected throughout treatment and post-treatment follow-up.

Results: 409 HCV Genotype 1 mono-infected patients were given a combination of Sofosbuvir (SOF: 400 mg/d) and Daclatasvir (DCV: 60 mg/d) without ribavirin (n = 318) or with ribavirin (1–1.2 g/d, n = 91). 318 patients had cirrhosis and 306 were previously treated with PR (n = 134) or PR + a first generation PI (n = 172).

The overall SVR4 rate was 81.6, 93.9, 100 and 96.6% in those who were given for 12 and 24 weeks SOF/DCV and SOF/DCV/RBV, respectively. The overall SVR4 rate differed according to the prior history and fibrosis (Table). The 12-week combination SOF/DCV/RBV achieved a 100% SVR4 rate in cirrhotics without additive effect of extension of the treatment to 24 weeks with or without RBV (95.7 and 92.5, respectively) and this was also true in experienced patients; all non-cirrhotic patients achieved 100% SVR4 at 12 weeks and, thus, the 12-week combination of SOF/DCV is a good therapeutic option. Serious adverse events were only reported in 9% and treatment discontinuation related to adverse events in 3.1%.

	SOF/DCV (n = 318)		SOF/DCV/RBV (n = 91)	
	12 weeks	24 weeks	12 weeks	24 weeks
% SVR4	81.6	93.9	100.0	96.6
% SVR12	86.8	94.0	100.0	100.0
% SVR4 cirrhotic	69.0	92.5	100.0	95.7
% SVR4 noncirrhotic	100.0	100.0	100.0	100.0
% SVR4 naïve	87.0	86.8	100.0	100.0
% SVR4 experienced	76.9	95.3	100.0	95.9

Conclusions: The Sofosbuvir/Daclatasvir combination is associated with a high rate of SVR4 in difficult-to-treat patients infected by Genotype 1. The combination of Ribavirin increases the SVR rate in cirrhotic or experienced patients without additive effect of the extension of the treatment from 12 to 24 weeks.

*The ANRS CO22 Hepather cohort is supported by MSD, Janssen, Gilead, BMS, Roche, Abbvie and conducted in collaboration with AFEF.

LO4

A PHASE 2, RANDOMIZED, PLACEBO-CONTROLLED STUDY (IMAGO) OF LUM001, A NOVEL INHIBITOR OF THE APICAL SODIUM-DEPENDENT BILE ACID TRANSPORTER (ASBT), IN PAEDIATRIC PATIENTS WITH ALAGILLE SYNDROME (ALGS)

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Introduction: Alagille syndrome (ALGS) is a rare, life-threatening genetic disorder characterized by cholestatic liver disease (CLD) and extrahepatic manifestations. Children with ALGS experience severe pruritus and markedly reduced quality of life and may require liver transplantation. Numerous pharmacological therapies have been tried, with limited success. Partial external biliary diversion (PEBD), which interrupts the enterohepatic circulation, can improve biochemical markers of cholestasis and ameliorate pruritus, but is disfiguring and can be associated with frequent complications. LUM001 is a potent, selective, minimally absorbed inhibitor of the apical sodium-dependent bile acid transporter (ASBT); it blocks bile acid reabsorption in the terminal ileum and increases faecal bile acid excretion, thereby reducing recirculation of bile acids to the liver. We report safety and efficacy data from IMAGO, the first study of LUM001 in children with ALGS.

Material and Methods: Children aged 1–18 years with ALGS, cholestasis (total serum bile acid [sBA] >3 times upper normal limit) and intractable pruritus, who had not undergone liver transplantation or PEBD, were randomized 2:1 to once-daily LUM001 (140 or 280 μg/kg/day) or placebo. LUM001 dosing started at 14 μg/kg/day and increased weekly for 5 weeks to reach the target dose in each group. The primary endpoint was change in fasting sBA level from baseline to Week 13. Secondary endpoints were change in pruritus, assessed twice daily with a novel reported outcome measure (the ItchRO) using a hand-held electronic diary, and liver enzymes (ALT, AST and ALP). Safety assessments included adverse events, laboratory values and vital signs. Comparisons between LUM001 and placebo were made using an analysis of covariance model and descriptive statistics.

Results: In total, 20 patients were enrolled in IMAGO, with 19 completing 13 weeks of therapy. All patients who completed IMAGO elected to enter a 72-week extension study (IMAGINE-I). Results from IMAGO will be available in early April.

Conclusions: This is the first report of the use of the novel pharmacological therapy LUM001 to inhibit ASBT for the treatment of CLD and severe pruritus in children with ALGS. The results of this study will have significant implications for ALGS treatment. LUM001 may substitute for PEBD, an invasive and disfiguring surgery, and prevent or delay the need for liver transplantation.

Study funded by Lumena (part of the Shire Group of Companies).

LO5

SOFOSBUVIR + PEGINTERFERON/RIBAVIRIN FOR 12 WEEKS VS SOFOSBUVIR + RIBAVIRIN FOR 16 OR 24 WEEKS IN GENOTYPE 3 HCV INFECTED PATIENTS AND TREATMENT-EXPERIENCED CIRRHOTIC PATIENTS WITH GENOTYPE 2 HCV: THE BOSON STUDY

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Introduction: Sofosbuvir (SOF) in combination with ribavirin with or without peginterferon (PEG) has demonstrated high efficacy in genotype 2 or 3 HCV-infected patients. However, these regimens have not been directly compared. The phase 3 BOSON study evaluated the safety and efficacy of SOF+PEG/RBV for 12 weeks vs SOF+RBV for 16 or 24 weeks in treatment-experienced genotype 2 (GT2) HCV-infected patients with cirrhosis, and in treatment-naïve and -experienced genotype 3 (GT3) HCV-infected patients with and without cirrhosis.

Material and Methods: Patients were randomized 1:1:1 to receive either SOF+RBV for 16 or 24 weeks or SOF+PEG/RBV for 12 weeks and stratified by HCV genotype and cirrhosis status. All patients received SOF 400 mg daily and RBV 1000–1200 mg in a divided daily dose. PEG was administered as 180 μg weekly injection. The primary end point was sustained virologic response 12 weeks after treatment (SVR12).

Results: Of 592 patients randomized and treated, 92% had GT3 HCV, 67% were male, 84% white, 53% treatment experienced, 62% had non-CC IL28B genotypes, and 37% had cirrhosis. GT2 treatment-experienced patients with cirrhosis had high SVR12 rates in all treatment groups: 87% of those receiving SOF+RBV for 16 weeks, 100% of those receiving SOF+RBV for 24 weeks, and 94% of those receiving SOF+PEG/RBV for 12 weeks. Among GT3 patients, SVR12 rates were highest in those receiving SOF+PEG/RBV for 12 weeks (93%) as compared to SOF+RBV for 24 (84%, p 0.008) or 16 weeks (71%, p < 0.001) (Table). The most common adverse events in all arms were fatigue, headache, insomnia, and nausea. Overall, 6 (1%) patients discontinued treatment due to adverse events; one of them was treated with SOF+PEG/RBV.

Table: SVR12 in GT3 patients

	SVR, % (n/N)		
	SOF+RBV 16 weeks N = 181	SOF+RBV 24 weeks N = 182	SOF+PEG/RBV 12 weeks N = 181
Overall	71% (128/181)	84% (153/182)	93% (168/181)
Treatment-naive	77% (70/91)	88% (83/94)	95% (89/94)
Cirrhotic	57% (12/21)	82% (18/22)	91% (21/23)
Non cirrhotic	83% (58/70)	90% (65/72)	96% (68/71)
Treatment-experienced	64% (58/90)	80% (70/88)	91% (79/87)
Cirrhotic	47% (17/36)	77% (26/34)	86% (30/35)
Non cirrhotic	76% (41/54)	82% (44/54)	94% (49/52)

Conclusions: GT2 treatment-experienced patients with cirrhosis had high SVR12 rates in all treatment arms. In GT3 patients, including a large proportion of treatment-experienced patients with cirrhosis, SOF+PEG/RBV for 12 weeks resulted in the highest SVR12 rates observed to date in a Phase 3 study. Overall and in all subgroups, GT3 patients receiving 24 weeks of SOF+RBV had higher SVR12 rates than those receiving 16 weeks of treatment, confirming that 24 weeks is the optimal duration for this combination in GT3 patients. SOF+PEG/RBV for 12 weeks was well tolerated with a high rate of treatment completion. These data suggest SOF+PEG/RBV treatment should still be considered for IFN-eligible GT3 patients, particularly for those with cirrhosis and/or prior treatment failure.

LO6

A TWO-STAGE GENOME-WIDE ASSOCIATION STUDY IDENTIFIES TM6SF2 AND MBOAT7 AS RISK LOCI FOR ALCOHOL-RELATED CIRRHOSIS

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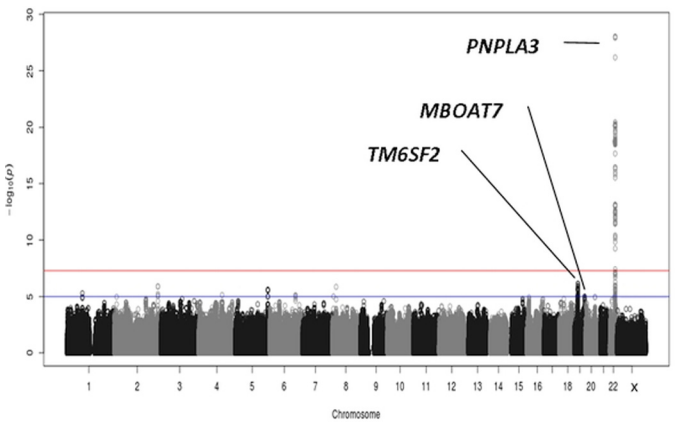
Introduction: Alcohol misuse is a major cause of cirrhosis in the Western world, but only <20% of alcohol misusers eventually develop significant liver disease. Multiple lines of evidence suggest that the risk of developing alcohol-related cirrhosis is genetically modulated. Nevertheless, the only robust, replicated finding to date is of a strong risk association with a genetic variant in *PNPLA3*, but no systematic genome-wide association studies (GWAS) of alcohol-related cirrhosis risk have been undertaken to date.

Material and Methods: A two-stage GWAS was performed comparing cases with alcohol-related cirrhosis with alcohol dependent controls with no evidence of liver disease. In the first stage, individuals recruited from Germany (410 cases *vs.* 1119 controls) and the UK (302 cases *vs.* 347 controls) underwent separate genome-wide association analysis followed by meta-analysis implemented in META v.1.5.0. In the second stage, the top

GWAS hits were validated in independent cohorts from Germany (529 cases *vs.* 761 controls) and Belgium (619 cases *vs.* 161 controls) and the results of the joint analyses combined. Genotyping was undertaken using Illumina BeadChips (Illumina Inc., San Diego, CA, USA); SNP replication was undertaken using Taqman chemistry (Applied Biosystems, Foster City, Ca, USA) on an automated platform.

Results: The strongest association signal in the initial meta-analysis was observed between the rs738409 variant in *PNPLA3* ($P_{meta} = 1.17 \times 10^{-28}$, OR = 2.38 [2.08–2.69]); 102 separate variants at the *PNPLA3* locus associated with genome-wide significance ($P_{threshold} < 5 \times 10^{-8}$). In addition, nine other independent loci provided borderline association signals ($P_{threshold} \leq 1.1 \times 10^{-5}$). Validation genotyping for rs738409 in *PNPLA3* and lead markers for the top 10 associated regions confirmed disease association for rs738409 in *PNPLA3*, and for (*MBOAT7:* rs641738 $P_{replication} = 1.35 \times 10^{-4}$; $P_{combined} = 9.25 \times 10^{-10}$; OR = 1.63 [1.46–1.80] and *TM6SF2:* rs10401969 $P_{replication} = 3.29 \times 10^{-5}$; $P_{combined} = 1.73 \times 10^{-8}$; OR = 1.35 [1.25–1.44]).



Conclusions: This first multicenter GWAS in alcoholic liver disease identifies variants within the genes coding for *PNPLA3, TM6SF2* and *MBOAT7* as significant risk loci for cirrhosis. All play a role in lipid metabolism suggesting that dysfunctional lipid signalling pathways – partly overlapping with non-alcoholic fatty liver disease – play an important role in the pathogenesis of alcohol-related cirrhosis.

LO7

A SINGLE SUBCUTANEOUS DOSE OF 2 mg/kg OR 4 mg/kg OF RG-101, A GalNAc-CONJUGATED OLIGONUCLEOTIDE WITH ANTAGONIST ACTIVITY AGAINST MIR-122, RESULTS IN SIGNIFICANT VIRAL LOAD REDUCTIONS IN CHRONIC HEPATITIS C PATIENTS

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Introduction: MicroRNA-122 (miR-122) is an important host factor for hepatitis C virus (HCV) replication. Binding of miR-122 to the HCV 5′-UTR RNA protects the genome from degradation and prevents induction of an innate immune response against the virus. RG-101 is a hepatocyte targeted carbohydrate conjugated oligonucleotide with potent antagonist activity against miR-122. The aim of this study was to evaluate the safety and efficacy of RG-101 in chronic HCV patients with various genotypes.

Material and Methods: In this multicenter phase 1 study, we included 32 chronic HCV patients with genotype 1, 3 or 4 infection. The first cohort of 16 patients received a single subcutaneous injection of 2 mg/kg RG-101 (n = 14) or placebo (n = 2), the second cohort received a single subcutaneous injection of 4 mg/kg (n = 14) or placebo (n = 2). Both cohorts were followed until 57 days after randomization, and patients with HCV RNA levels below the limit of quantification (BLOQ) at day 57 were included in an extended follow-up study. HCV RNA levels were measured using Roche COBAS AmpliPrep/COBAS Taqman HCV v2.0 assay, with a reported LLOQ of 15 IU/mL.

Results: Thirty-two patients infected with HCV genotype 1 (n = 16), genotype 3 (n = 10) or genotype 4 (n = 6) were included. Twenty-three patients were treatment naive and 9 patients were virological relapsers to a prior interferon-based therapy. None of the patients had cirrhosis. At baseline, mean HCV RNA levels were comparable between RG-101 dosed patients versus placebo (6.2 versus 6.4 log 10 IU/mL, p = 0.53). The mean viral load reduction at day 29 was 4.1 log10 IU/mL (range 2.3–5.8) in patients dosed with 2 mg/kg, and 4.8 log10 IU/mL (range 3.0–5.8) in patients dosed with 4 mg/kg RG-101, as compared with a reduction of 0.0 in the placebo group (p < 0.001). At day 57, 15/28 patients (gt 1 n = 6, gt 3 n = 5, gt 4 n = 4) dosed with RG-101 had HCV RNA levels BLOQ, and 12 of these 15 patients had HCV RNA levels that could not be detected. In 10/15 patients (gt 1 n = 3, gt 3 n = 3, gt 4 n = 4) HCV RNA levels remained BLOQ at day 85. No dose-limiting adverse events were observed and none of the patients discontinued the study.

Conclusions: A single administration of 2 mg/kg or 4 mg/kg RG-101 was well tolerated and resulted in significant reductions in HCV RNA levels in patients infected with various chronic HCV genotypes and prior treatment history. Patients with HCV RNA levels BLOQ will be followed up to six months to assess if viral cure can be established with one single administration of RG-101.

LO8

DACLATASVIR, SOFOSBUVIR, AND RIBAVIRIN COMBINATION FOR HCV PATIENTS WITH ADVANCED CIRRHOSIS OR POSTTRANSPLANT RECURRENCE: PHASE 3 ALLY-1 STUDY

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Introduction: The pangenotypic combination of daclatasvir (DCV) and sofosbuvir (SOF) achieves high rates of SVR in patients with chronic HCV infection. DCV+SOF has favorable safety and drug interaction profiles and a high resistance barrier. These attributes support the ALLY-1 study of DCV+SOF with ribavirin (RBV) in patients with advanced cirrhosis or post-liver transplant HCV recurrence, who have a high unmet therapeutic need.

Material and Methods: This open-label study enrolled treatment-naive or experienced adults with HCV infection of any genotype (GT) in 2 cohorts: (1) advanced cirrhosis, (2) post-liver transplant recurrence. Patients received 12 weeks of treatment with once-daily DCV 60 mg + once-daily SOF 400 mg and RBV (initially 600 mg/d, adjusted for hemoglobin and creatinine clearance). In the cirrhosis cohort, patients transplanted during treatment could receive 12 weeks of extended treatment immediately posttransplant,

regardless of treatment duration before transplant. The primary endpoint was HCV RNA <LLOQ (25 IU/mL) at posttreatment Week 12 (SVR12) in patients with GT1 in each cohort.

Results: The cirrhosis (N = 60) and posttransplant (N = 53) cohorts were, respectively, 40% and 42% treatment-naive and 75% and 77% GT1. The Child–Pugh class in the cirrhosis cohort was 20% A, 53% B, and 27% C. MELD score ranged from 8 to 27. No posttransplant patients had cholestatic recurrence or hepatic decompensation. Overall, 83% of patients in the cirrhosis cohort achieved SVR12, with higher SVR12 rates in patients with Child–Pugh class A or B disease than in those with class C (Table). In the posttransplant cohort, 94% achieved SVR12. Twelve of the 13 patients without SVR12 relapsed posttreatment. SVR12 rates were comparable regardless of prior treatment experience or baseline demographic characteristics. Four cirrhotic patients received a liver transplant during treatment; 3 of 4 extended treatment posttransplant and all 4 achieved SVR12. The most common AEs (any grade) were headache, fatigue, anemia, diarrhea, and nausea. There were no treatment-related serious AEs. One posttransplant patient discontinued all therapy after 31 days due to headache but achieved SVR12.

	SV12, % (n/N)	
	Advanced cirrhosis cohort	Posttransplant cohort
All patients	83 (50/50) [a,b]	94 (50/53)
GT 1 [95% CI]	82 (37/45) [c] [68, 92]	95 (39/41) [c] [84, 99]
GT 1a	76 (26/34)	97 (30/31)
GT 1b	100 (11/11)	90 (9/10)
GT 1, Child–Pugh A	91 (10/11)	–
GT 1, Child–Pugh B	92 (22/24)	–
GT 1, Child–Pugh C	50 (5/10)	–
GT 3	83 (5/6) [a]	91 (10/11)
GT 2, 4, or 6	89 (8/9) [b]	100 (1/1)

[a] Includes 1 patient (GT3) who had SVR12 documented after database lock.
[b] Includes 1 patient (GT4) who discontinued after 3 weeks for liver transplantation and achieved SVR12 off study.
[c] Primary endpoints.

Conclusions: DCV+SOF+RBV for 12 weeks was safe and well tolerated in both cohorts. SVR12 rates were >90% in patients with Child–Pugh class A or B cirrhosis but lower in Child–Pugh class C. SVR12 was achieved by 94% of liver transplant recipients with HCV recurrence.

EASL JOURNAL OF HEPATOLOGY

Thursday, 23 to Saturday, 25 April 2015

Late breaking abstracts – Posters

LP01

DACLATASVIR PLUS SOFOSBUVIR FOR TREATMENT OF HCV GENOTYPES 1–4 IN HIV–HCV COINFECTION: THE ALLY-2 STUDY

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Introduction: Daclatasvir (DCV; NS5A inhibitor) and sofosbuvir (SOF; nucleotide NS5B inhibitor) are potent, pangenotypic, well-tolerated, once-daily, oral HCV antivirals with limited pharmacokinetic interactions with other agents. DCV+SOF has demonstrated high rates of sustained virologic response (SVR) in HCV monoinfection. Efficacy and safety of DCV+SOF in HIV–HCV coinfection was assessed in a phase 3 study.

Material and Methods: ALLY-2 was a randomized, open-label study in HCV treatment-naive (n = 151) or -experienced (n = 52) adults coinfected with HIV and HCV (any genotype). Naive patients were randomized 2:1 to receive 12 or 8 weeks of SOF 400 mg + DCV 60 mg (dose adjusted for concomitant combination antiretroviral therapy (cART): 30 mg with ritonavir-boosted protease inhibitors [PI], 90 mg with nonnucleoside reverse transcriptase inhibitors [NNRTI] except rilpivirine). Experienced patients received DCV+SOF for 12 weeks. Primary endpoint was SVR at posttreatment week 12 (SVR12) in treatment-naive GT-1 patients who received 12 weeks of DCV+SOF.

Results: Patients were 87% male, 62% white, 34% black, and 14% cirrhotic, had a median age of 52 years, and were infected with HCV GT-1 (83%), GT-2 (9%), GT-3 (6%), or GT-4 (2%). Median baseline (BL) HCV RNA was 6.7 $\log_{10}$ IU/mL and median BL CD4 count was 565 cells/μL. Nearly all patients (98%) were on cART: 50% PI based, 25% NNRTI based, and 25% other regimens (primarily integrase inhibitors). A total of 98% of patients completed study treatment. Among GT-1 patients, SVR12 was achieved by 96% of naive and 98% of experienced patients after 12 weeks of DCV+SOF and by 76% of naive patients after 8 weeks; SVR12 rates for non-GT-1 patients in these groups were 100%, 100%, and 78%, respectively. SVR12 was similar in patients with or without cirrhosis and across other demographic and disease subgroups (Table). There were no HCV virologic breakthroughs, and HIV control was not compromised throughout the study period. Posttreatment HCV relapse occurred in 1–2% of patients in the 12-week treatment groups and 20% in the 8-week group. There were no treatment-related serious AEs or discontinuations for AEs.

		SVR12, % (n/N)		
		12 wk naive	12 wk experienced	8 wk naive
All patients [95% CI]		97 (98/101)	98 (51/52)	76 (38/50)
		[89.8, 99.2]	[88.0, 99.9]	[59.7, 87.6]
GT 1		96 (80/83)[a]	98 (43/44)	76 (31/41)
GT 1a		96 (68/71)	97 (32/33)	80 (28/35)
GT 1b		100 (12/12)	100 (11/11)	50 (3/6)
GT 2		100 (11/11)	100 (2/2)	83 (5/6)
GT 3		100 (6/6)	100 (4/4)	67 (2/3)
GT 4		100 (1/1)	100 (2/2)	–
Baseline HCV RNA	<6 million IU/mL	97 (56/58)	100 (33/33)	79 (27/34)
	≥6 million IU/mL	98 (42/43)	95 (18/19)	69 (11/16)
Cirrhosis	No	98 (88/90)	100 (34/34)	77 (34/44)
	Yes	89 (8/9)	93 (14/15)	60 (3/5)
Gender	Male	98 (90/92)	98 (42/43)	79 (33/42)
	Female	89 (8/9)	100 (9/9)	63 (5/8)
Age	<65 y	97 (93/96)	98 (48/49)	77 (36/47)
	≥65 y	100 (5/5)	100 (3/3)	67 (2/3)
Race	White	96 (63/66)	100 (31/31)	71 (20/28)
	Black	100 (30/30)	95 (19/20)	79 (15/19)
IL28B	CC	100 (28/28)	100 (13/13)	69 (9/13)
	non-CC	96 (70/73)	97 (38/39)	78 (29/37)
cART	PI	98 (46/47)	96 (22/23)	72 (21/29)
	NNRTI	100 (28/28)	100 (12/12)	80 (8/10)
	Other	92 (23/25)	100 (16/16)	78 (7/9)
Baseline CD4	<200 c/mm^3	100 (4/4)	–	100 (1/1)
	200–499 c/mm^3	98 (41/42)	100 (12/12)	71 (15/21)
	≥500 c/mm^3	96 (53/55)	100 (39/39)	79 (22/28)

c, cells: cART, combination antiretroviral therapy: CI, confidence interval; GT, genotype: NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
[a] Primary endpoint.

Conclusions: Treatment of HIV–HCV coinfected patients with DCV+SOF once daily for 12 weeks resulted in an overall 97% SVR12, and was well tolerated. DCV+SOF was effective in cirrhotics, in other demographic and disease categories, and across a broad range of cART regimens without compromising HIV virologic control.

LP02

C-SURFER: GRAZOPREVIR PLUS ELBASVIR IN TREATMENT-NAIVE AND TREATMENT-EXPERIENCED PATIENTS WITH HEPATITIS C VIRUS GENOTYPE 1 INFECTION AND CHRONIC KIDNEY DISEASE

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Introduction: HCV infection in patients with chronic kidney disease stages 4 and 5 (CKD4/5), defined as CrCl <30 mL/min ± dialysis-dependence, is associated with an increased risk of death and

kidney transplant failure. HCV-infected patients with CKD4/5 have limited HCV treatment options. Ribavirin-containing regimens are poorly tolerated; other treatments rely on renal clearance so are unsuitable. In a Phase 2 trial, grazoprevir (GZR; NS3/4A protease inhibitor) and elbasvir (EBR; NS5A inhibitor) was highly efficacious in treating HCV genotype 1 (G1) infection. Both medicines are cleared by the liver. We conducted a Phase 3 trial of GZR/EBR in HCV G1-infected patients with CKD4/5.

Material and Methods: 224 HCV G1-infected patients with CKD4/5 were randomized to immediate treatment with GZR 100 mg / EBR 50 mg once daily for 12 weeks or deferred treatment (placebo then active dosing). The trial also included an open-label GZR/EBR arm in which 11 patients underwent intensive pharmacokinetic (PK) sampling. The primary end point was sustained virologic response at follow-up week (FUW) 12 (SVR12; COBAS TaqMan v2.0 [LoQ 15 IU/mL]). Given the morbidity of CKD4/5, the primary efficacy analysis was done in a modified full analysis set (mFAS) population (patients in the immediate and intensive PK arms who received ≥1 dose of study drug, excluding those who died or discontinued for reasons unrelated to study drug). Safety was evaluated in the GZR/EBR arms and the placebo phase of the deferred arm.

Results: 235 patients received ≥1 dose of study drug (immediate, n = 111; PK, n = 11; deferred, n = 113): 52% had G1a infection, 80% were HCV treatment-naive; 6% were cirrhotic, 73% were male, 46% were African American, 34% had diabetes, 19% had CKD4, 81% had CKD5, and 76% were on hemodialysis. 5/122 (4.1%) patients in the GZR/EBR arms discontinued prior to FUW12 and were excluded from the mFAS population (vs. 6/113 [5.3%] in the deferred [placebo] arm). SVR12 was achieved in 99% (114/115) of patients in the active arms: 1 noncirrhotic patient relapsed at FUW12. Serious AEs occurred in 16 (14%) and 17 (15%) patients in the GZR/EBR and deferred arms, respectively. 0% and 4% of patients in the active and placebo groups, respectively, discontinued therapy due to an AE. The most common AEs in the active arms were headache, nausea, and fatigue.

Conclusions: Once-daily GZR/EBR for 12 weeks was highly effective and generally well tolerated in patients with HCV G1 infection and advanced CKD.

LP03

SAFETY AND EFFICACY OF SHORT-DURATION TREATMENT WITH GS-9857 COMBINED WITH SOFOSBUVIR/GS-5816 IN TREATMENT-NAÏVE AND DAA-EXPERIENCED GENOTYPE 1 PATIENTS WITH AND WITHOUT CIRRHOSIS

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Introduction: GS-9857, a potent pan-genotypic HCV NS3/4A protease inhibitor (PI) with an enhanced resistance profile compared to first generation HCV PIs, demonstrated >3 log reductions in HCV RNA after 3 days of monotherapy in a Phase 1b study in patients infected with HCV genotypes 1–4. Here, we report the safety and efficacy of GS-9857 with SOF/GS-5816, a fixed dose combination of pangenotypic NS5B and NS5A inhibitors, in a regimen with 3 different mechanisms of action for 4 or 6 weeks in treatment-naïve and direct-acting antiviral (DAA)-experienced patients with HCV genotype 1 with and without cirrhosis.

Material and Methods: Treatment-naïve genotype 1 HCV-infected patients without cirrhosis were treated for 4 or 6 weeks with GS-9857 with SOF/GS-5816. Treatment-naïve genotype 1 HCV-infected patients with cirrhosis and those who had failed ≥2 DAAs were enrolled in separate cohorts to also receive 6 weeks of GS-9857 with SOF/GS-5816. HCV RNA was measured using the COBAS® AmpliPrep/COBAS® Taqman® assay V2.0 with a lower limit of quantification (LLOQ) of 15 IU/mL.

Results: 75 patients with HCV genotype 1 were enrolled. The majority were male (51, 68%), had subtype 1a infection (59, 79%), and had the IL28B non-CC genotype (51, 68%). Median HCV RNA in each group ranged from 6.31 to 6.46 log10 IU/mL. All patients completed treatment and 97% achieved rapid viral suppression to <LLOQ by Week 4. SVR4, SVR12 and relapse data are presented in the table. There were no Grade 3 or 4 adverse events, serious adverse events, or deaths. The most frequent adverse events were nausea (19, 25%), headache (18, 24%), and fatigue (12, 16%). Elevated lipase was the only grade 3 or 4 laboratory abnormality that occurred in more than one patient (3, 4%).

Table: Patients with HCV Genotype 1 infection with SVR4, SVR12 and Relapse

Treatment group	Treatment-naïve			Prior DAA failure
	No cirrhosis		Cirrhosis	20% Cirrhosis
	4 weeks	6 weeks	6 weeks	6 weeks
N	15	15	15	30
SVR4, n (%)	11 (73)	15 (100)	12 (80)	26 (87)
SVR12, n (%)	3 (20)	14 (93)	12 (80)	Not available
Relapse, n (%)	11 (73)	1 (7)	2 (13)	4 (13)

Conclusions: GS-9857 with SOF/GS-5816 was well tolerated for 4 or 6 weeks. Treatment for 6 weeks was highly efficacious in GT1 treatment-naïve patients without cirrhosis. Treatment-experienced patients or those with cirrhosis may benefit from longer durations of therapy. Ongoing Phase 2 studies are evaluating GS-9857 with SOF/GS-5816 for durations of 6, 8 and 12 weeks in genotype 1–6 patients.

LP04

A PHASE 3, OPEN-LABEL, SINGLE-ARM STUDY TO EVALUATE THE EFFICACY AND SAFETY OF 12 WEEKS OF SIMEPREVIR (SMV) PLUS SOFOSBUVIR (SOF) IN TREATMENT-NAÏVE OR -EXPERIENCED PATIENTS WITH CHRONIC HCV GENOTYPE 1 INFECTION AND CIRRHOSIS: OPTIMIST-2

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Introduction: Hepatitis C virus (HCV)-infected patients (pts) with cirrhosis are historically a difficult-to-treat pt population. In a Phase II study (COSMOS), high sustained virologic response 12 weeks (wks) after end of treatment (EOT; SVR12) rates were achieved in METAVIR F4 treatment-naïve and prior null responder pts treated with an all oral, once-daily (QD) combination of SMV (HCV NS3/4A protease inhibitor)+SOF (HCV nucleotide-analogue NS5B polymerase inhibitor) for 12 or 24 wks, regardless of the presence or absence of ribavirin. The OPTIMIST-2 (NCT02114151) study aimed to demonstrate superiority of 12 wks of SMV+SOF, in treatment-naïve or -experienced (including interferon (IFN)-intolerant) HCV genotype (GT)1-infected pts with cirrhosis compared with a

historical control (composite of the SVR12 rates of approved direct-acting antiviral/IFN+ribavirin regimens).

Material and Methods: Treatment-naïve or -experienced pts with chronic HCV GT1 infection and documented presence of cirrhosis received SMV 150 mg QD+SOF 400 mg QD for 12 wks. The primary efficacy endpoint was SVR12 in the overall population. Safety and pt-reported outcomes were assessed.

Results: 103 pts received treatment (male, 81%; median age, 58 years; Black/African American, 18%; IL28B CC, 28%; GT1a/1b, 70/30%; treatment-naïve [n = 50, 49%]; treatment-experienced [n = 53, 52%]). SVR12 with SMV+SOF (84% [95% confidence interval: 76, 91]) met the primary endpoint of superiority to the historical control (70%). Other endpoints are summarised (Table 1). Adverse events (AEs) were observed in 72 (70%) pts, these were mainly Grade 1/2 (64%). Most frequent AEs: fatigue (20%), headache (20%) and nausea (11%). AEs of interest, increased bilirubin and rash, occurred in 2 (2%) and 16 (16%) pts, respectively. Three (3%) pts discontinued at least 1 drug due to an AE. Serious AEs were infrequent (5 pts [5%]). One pt died in a motor vehicle accident. Pt-reported outcomes improved from baseline to follow-up wk 12 with clinically important improvements in quality of life for pts who achieved SVR12 (Table 2). Additional data will be presented.

Table 1. Results (overall and sub-groups) with 12 weeks of SMV 150 mg QD + SOF 400 mg QD

	n/N (%)
SVR12 [a]	86/103 (84)
Treatment-naïve	44/50 (88)
Treatment-experienced	42/53 (79)
HCV GT1a	60/72 (83)
HCV GT1b	26/31 (84)
IL28B CC	25/29 (86)
IL28B non-CC	61/73 (84)
On-treatment failure [b]	3/103 (3)
Viral relapse [c]	13/103 (13)

[a] HCV RNA <25 IU/mL undetectable 12 wks after EOT.
[b] HCV RNA <25 IU/mL detectable or ≥25 IU/mL at EOT.
[c] HCV RNA <25 IU/mL undetectable at EOT & ≥25 UI/mL during follow-up.

Table 2. Patient-reported outcomes

Patient-reported outcome	Mean (SD) change from baseline	
	Patients with SVR12 (n = 79)	Patients without SVR12 (n = 13)
HCV-SIQv4 OBSS [d]	−5.9 (1.5)	−5.3 (2.9)
FSS [e]	−0.6 (0.2)	−0.7 (0.4)
CES-D [f]	−3.5 (1.1)	−3.5 (1.9)
EQ-5D VAS [g]	10.8 (2.1)	3.7 (5.1)

[d] HCV-SIQv4 OBSS, Hepatitis C Symptom & Impact Questionnaire version 4 Overall Body System Score (range 0 [no symptoms] to 100 [maximum severity for all 29 symptoms]; mean reduction of ≥5 points is clinically important).
[e] FSS, Fatigue Severity Scale (range 1 [no fatigue] to 7 [severe fatigue]; mean reduction of 0.6 points is clinically important).
[f] CES-D, Center for Epidemiologic Studies Depression Scale (range 0 [no depressive symptoms] to 60 [severe depression on 20 symptoms]; mean reduction of ≥3 points is clinically important).
[g] EQ-5D VAS, EuroQol-5 Dimension questionnaire Visual Analogue Scale (range 0 [worst possible health] to 100 [perfect health]; mean increase of ≥5 points is clinically important).

Conclusions: SMV+SOF for 12 wks achieved superiority in SVR12 rates vs the historical control in treatment-naïve and -experienced HCV GT1-infected pts with cirrhosis and was generally well tolerated.

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LP05

DACLATASVIR PLUS SOFOSBUVIR WITH OR WITHOUT RIBAVIRIN IN PATIENTS WITH HCV GENOTYPE 3 INFECTION: INTERIM ANALYSIS OF A FRENCH MULTICENTER COMPASSIONATE USE PROGRAM

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Introduction: Treatment options for HCV genotype 3 (GT3) patients are limited. The combination daclatasvir (DCV) and sofosbuvir (SOF) for 12 weeks is associated with high SVR rate in genotype 3 non cirrhotic patients (96% SVR12) and a lower response in cirrhotic (63% SVR12). GT3 cirrhotic remain a difficult to treat population and may benefit from the addition of ribavirin (RBV) or extended treatment duration. This analysis reports interim results from a French multicenter compassionate use program of DCV+SOF±RBV in patients with HCV genotype 3 chronic infection.

Material and Methods: The ATU has been managing over 4000 HCV patients from 221 French centers. Patients received DCV+SOF QD for 12 or 24 weeks, with RBV added at the physician's discretion.

	DCV/SOF +/− RBV			
	12 weeks		24 weeks	
	cirrhotic	Non cirrhotic	cirrhotic	Non cirrhotic
SVR4	22/29 (76%)	11/12 (92%)	52/59 (88%)	5/6 (83%)

Results: 601 HCV genotype 3 patients with severe fibrosis (F3) or cirrhosis (F4), or HCV extrahepatic manifestations or post-liver transplant HCV recurrence or indication for liver or kidney transplantation enrolled in the program. Most patients were male (75%), HCV mono-infected (83%), cirrhotic (77%), and treatment experienced (73%). The median age was 54.3 years (27–83). 64% and 15% planned to receive DCV+SOF for 24 weeks with or without RBV, respectively, 4% and 17% planned to receive DCV+SOF for 12 weeks with or without RBV, respectively. Baseline median HCV RNA level was 6.07 (1.20–7.62) log10 IU/mL, platelets count 118.5x10^9/L (31–387) and albumin was 39.0 g/L (13–56). Treatment discontinuations were related to adverse event in 1 patient, death in 2 patients and patient's decision in 1 patient. The table below shows the interim results in the first 106 patients without liver transplant who

reached the week 4 post-treatment visit according to treatment schedule, fibrosis stage and patient status. Efficacy and safety data will be updated in larger population.

Conclusions: This preliminary analysis is consistent with previous findings and demonstrates that12 weeks of DCV+SOF in GT3 patients results in a high SVR4 in non cirrhotic patient population. Cirrhotic patients appeared to benefit from the extended treatment duration to 24 weeks.

LP06

SUSTAINED VIROLOGIC RESPONSE AFTER ACH-3102 AND SOFOSBUVIR TREATMENT FOR 8 OR 6 WEEKS: A PHASE 2 "PROXY" STUDY

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Introduction: ACH-3102 is a 2nd generation NS5A inhibitor in Phase 2 development for the treatment of chronic hepatitis C viral (HCV) infection. ACH-3422, a uridine nucleotide HCV NS5B polymerase inhibitor in Phase 1, is planned for use in combination with ACH-3102. This Phase 2, open-label, randomised, controlled, partial crossover study investigates the efficacy and safety of ACH-3102 plus sofosbuvir (used as a proxy for ACH-3422). Interim and final SVR data from patients (pts) receiving 6-week (wk) and 8-wk treatment durations, respectively, are reported.

Material and Methods: A total of 30 pts with chronic genotype (GT)-1 HCV infection were enrolled in two groups. In the 8-wk treatment group, 12 pts were randomised to active treatment with ACH-3102 (50 mg) and sofosbuvir (400 mg) once daily, and 6 pts were randomised to observation. Based on favorable SVR4 results from this group, a 6-wk treatment group was initiated with the same active treatment. The 6 observational pts from the 8-wk treatment group were assigned to active treatment, 6 new pts were randomised to active treatment, and 6 new pts were randomized to observation.

Results: Of active pts in the 8- and 6-wk treatment groups, 83% and 58% were infected with GT-1a HCV, 75% and 58% were male, and mean ages were 42.6 and 49.2 years, respectively. Baseline viral load in active pts from the 8- and 6-wk treatment groups ranged from 5.5–8.0 $\log_{10}$IU/mL, with 9 and 7 pts in each group, respectively, with values >6,000,000 IU/mL. SVR24 was 100% in the 8-wk treatment group. All active pts in the 6-wk group achieved HCV RNA levels below the limit of quantification by Week 4 and below the limit of detection by Week 6 (end of treatment). The incidence of SVR12 in the 6-wk treatment group was 100%. Treatment was well-tolerated in both groups, with no serious adverse events and no discontinuations.

Conclusions: ACH-3102 is a potent NS5A inhibitor that achieved 100% SVR12 when combined with sofosbuvir in pts with chronic GT-1 HCV infection. Future studies will explore use of ACH-3102 in sofosbuvir-containing regimens for treatment durations as short as 4 weeks, and will also explore sofosbuvir "sparing" regimens using a reduced number of sofosbuvir doses. A parallel development track will explore the combination of ACH-3102 and ACH-3422 +/– sovaprevir (an NS3 protease inhibitor) in interferon- and ribavirin-free regimens with short treatment durations (4–8 weeks) across different patient populations.

LP07

SIMEPREVIR (SMV) PLUS DACLATASVIR (DCV) AND SOFOSBUVIR (SOF) IN TREATMENT-NAÏVE AND -EXPERIENCED PATIENTS WITH CHRONIC HEPATITIS C VIRUS GENOTYPE 1 OR 4 INFECTION AND DECOMPENSATED LIVER DISEASE: INTERIM RESULTS FROM THE PHASE II IMPACT STUDY

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Introduction: Interim analysis (IA) data from IMPACT (NCT02262728), an ongoing Phase II open-label study assessing for the first time an all-oral regimen of SMV (HCV NS3/4A protease inhibitor), in combination with DCV (HCV NS5A replication complex inhibitor) and SOF (HCV nucleotide-analogue NS5B polymerase inhibitor) in chronic HCV genotype (GT)1/4-infected patients (pts) with decompensated liver disease, a population with a high medical need and limited therapeutic options.

Material and Methods: HCV treatment-naïve or (peg)IFN±ribavirin treatment-experienced GT1/4-infected cirrhotic pts ≥18 yrs with Child–Pugh (CP) score <7 (Class A) with evidence of portal hypertension or 7–9 (Class B) were enrolled. Pts received 12 wks of SMV (150 mg QD), DCV (60 mg QD) and SOF (400 mg QD). Primary efficacy endpoint: sustained virologic response 12 wks after end of treatment (EOT, SVR12). Key secondary endpoints: SVR4, on-treatment failure, viral relapse, PK and safety.

Table: Plasma PK parameters

	Mean (SD)			
	Week 2		Week 8	
	Child–Pugh A (n = 19)	Child–Pugh B (n = 9)	Child–Pugh A (n = 18)	Child–Pugh B (n = 9)
Simeprevir				
C_{max} (ng/mL)	6976 (4439)	7951 (4676)	6029 (3936)	8841 (5384)
AUC (h·ng/mL)	113873 (91579)	144310 (88836)	98561 (80028)	176091 (114832)[a]
Daclatasvir				
C_{max} (ng/mL)	1152 (414)	958 (420)	1072 (313)	854 (427)
AUC (h·ng/mL)	16022 (6470)	14250 (7352)	15574 (5342)	13530 (6662)
Sofosbuvir				
C_{max} (ng/mL)	1571 (738)[b]	1743 (1331)	1276 (856)	1418 (977)
AUC (h·ng/mL)	2864 (978)[b]	3768 (1817)	2729 (988)	3769 (1800)

AUC, area under the concentration–time curve; C_{max}, maximum plasma concentration.
[a] n = 8. [b] n = 18.

Results: IA in 28 pts who received treatment (male 64.3%; median age 58.0 yrs; White 96.4%; GT1a/1b/4 71.4/25.0/3.6%; treatment-naive/experienced 50/50%; Fibroscan median score 26.7 kPa [range 14.9–63.9]; CP class A/B 19/9 pts). Baseline Q80K was present in 11/20 GT1a pts and 0 GT1b/GT4 pts. 28 pts reached EOT; 14 reached Wk4 follow-up. Virologic response rates (HCV RNA <15 IU/mL) for CP A/B were 89.5/33.3% at Wk2, 100/77.8% at Wk4 and 100/100% at Wks 8, 10 and 12. All pts with available data achieved SVR4 (CP A 12/12; CP B 2/2). Wk2 mean SMV exposure (AUC) was 1.3 fold higher in CP B vs CP A pts, but individual pt exposures in the CP B group were within the range observed for CP A. Mean SMV exposures in both groups were comparable for Wk2 vs Wk8 (Table). Mean DCV and SOF exposures were each similar within groups and at Wk2 vs Wk8. Adverse events (AEs) occurred in 57.1% of pts (CP A/B 47.4/77.8%); all but one were Grade 1/2 at the time of analysis (1 serious AE [sick sinus syndrome; unrelated to study medication] reported 1 wk after EOT). Most common AEs: pruritus (3 events; CP A/B 1/2) and urinary tract infection (2 events; CP A/B 1/1). At the time of analysis, there were no deaths or discontinuations due to

AEs. Laboratory abnormalities were generally Grade 1/2. Additional data will be presented.

Conclusions: In this Phase II study, the combination of SMV+DCV+SOF resulted in high on-treatment virologic response and SVR4 rates, and was safe and well tolerated in decompensated liver disease pts.

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LP08

SAFETY AND EFFICACY OF SOFOSBUVIR-CONTAINING REGIMENS IN HEPATITIS C INFECTED PATIENTS WITH REDUCED RENAL FUNCTION: REAL-WORLD EXPERIENCE FROM HCV-TARGET

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Introduction: Renal clearance is the major elimination pathway for GS331007, the major metabolite of SOF, and pharmacokinetic studies show higher drug and metabolite concentrations in those with estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2. We assessed the safety and efficacy of SOF-containing regimens in HCV infected patients with varying baseline renal function.

Material and Methods: HCV-TARGET is a longitudinal observational study of patients treated with direct-acting antivirals at academic (n = 43) and community (n = 13) medical centers in North America (n = 51) and Europe (n = 5) with centralized data abstraction and independent data monitoring. Demographic, clinical, adverse events (AEs), and virological data are collected throughout treatment and post-treatment follow-up from enrolled patients. Patients with baseline eGFR (Modification of Diet in Renal Disease equation) ≤30 vs. 31–45 vs. 46–60 vs. >60 mL/min/1.73m^2 were examined.

Table: Treatment regimen, safety and efficacy outcomes by baseline MDRD eOFR

	eGFR ≤30 (n = 18)	eGFR 31–45 (n = 63)	eGFR 46–60 (n = 168)	eGFR >60 (n = 1,641)	p-value
Treatment Regimen, %					
SOF/PEG/RBV	6	6	9	20	<0.01
SOF/RBV	22	27	35	32	0.61
SOF/SMV	61	51	47	37	<0.01
SOF/SMV/RBV	11	16	10	11	0.57
Common SOF Adverse Events (AE), %					
Fatigue	19	37	33	35	0.58
Headache	6	17	12	18	0.22
Nausea	19	15	19	16	0.71
Anemia AE, %	31	28	23	15	<0.01
Required transfusion(s), %	13	9	1	2	<0.01
Received erythropoietin, %	0	11	8	3	<0.01
Reduction in RBV due to anemia, %	50	27	30	15	<0.01
RBV discontinuation, %	0	12	1	1	<0.01
Acute renal insufficiency [a], %	25	13	2	1	<0.01
Renal or urinary system AE [b], %	25	13	8	5	<0.01
Serious Adverse Event, %	19	24	5	6	<0.01
Early treatment discontinuation, %	9	8	4	4	0.23
due to AE, %	6	5	2	2	0.25
Death, %	6	0	1	0.6	0.11
SVR12 (n = 1,457), %	85 (11/13)	81 (30/37)	88 (108/123)	84 (1,075/1,284)	0.65

PEG, peg-interferon; SMV, simeprevir.

[a] Includes acute on chronic renal insufficiency. Outcome abstracted from treatment documentation.

[b] Includes acute renal failure, dysuria, hematuria, urinary retention and other similar renal/urinary problems.

Results: 1,890 patients [genotype 1 (71%), 2 (17%), 3 (9%), 4–6 (3%)], of which 129 (7%) are still undergoing therapy, with complete data for baseline eGFR calculation were included: 18 with eGFR ≤30 (5 on dialysis), 63 with eGFR 31–45, 168 with eGFR 46–60 and 1,641 with eGFR >60. Patients with baseline eGFR ≤30 (vs 31–45, 46–60, >60) differed in frequency of being female (72% vs 46%, 46%, 35%), age ≥65 (28% vs 29%, 32%, 18%), of Black race (22% vs 19%, 7%, 12%), having cirrhosis with decompensation (75% vs 69%, 65%, 45%), being post-liver transplant (39% vs 54%, 35%, 8%) and/or being post-kidney transplant (17% vs 8%, 5%, 1%) (all $p < 0.05$). Treatment regimens, safety and efficacy outcomes by baseline eGFR are shown in Table. All patients with eGFR <60 started with SOF 400 mg/day and had standard ribavirin (RBV) dose adjustments. Sustained virologic response (SVR) frequencies were similar across eGFR groups, ranging from 81–88% (Table). The one patient with baseline eGFR ≤30 died from worsening renal failure and hepatic decompensation while on therapy. When limited to patients on RBV-free regimens (n = 839), eGFR ≤30 patients still more frequently experienced anemia, acute renal insufficiency, renal or urinary system AEs and serious AEs (all $p < 0.05$).

Conclusions: Frequencies of SVR with SOF-containing regimens are high and not influenced by renal impairment. However, patients with reduced baseline renal function have a higher frequency of anemia, worsening renal dysfunction and serious adverse events regardless of use of RBV. Close monitoring during treatment is warranted.

LP09

HIGH EFFICACY OF RETREATMENT WITH LEDIPASVIR AND SOFOSBUVIR IN HCV PATIENTS WHO FAILED INITIAL SHORT COURSE THERAPY WITH COMBINATION DAA REGIMENS (NIH SYNERGY TRIAL)

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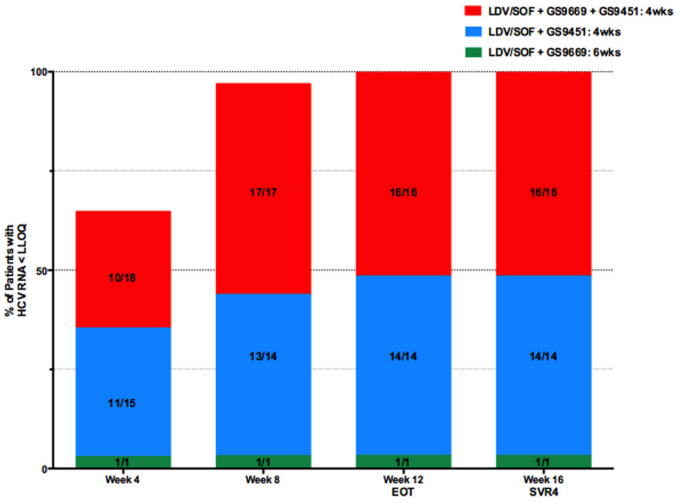
Introduction: Directly-acting antivirals (DAAs) have dramatically improved the treatment of Hepatitis C but retreatment strategies for patients who have failed to achieve sustained virologic response (SVR) with combination DAAs have not been studied. The aim of the current study is to determine if HCV genotype-1 patients who have failed short course combination therapy with three or four DAAs can be successfully treated with 12 weeks of ledipasvir (LDV) and sofosbuvir (SOF) in fixed dose combination (FDC).

Material and Methods: In this single-center, open-label, phase 2a trial, HCV mono-infected participants with early stage (F0-F2) liver fibrosis, with previous exposure to combination DAA therapy only (either LDV/SOF in combination with GS-9451 and/or GS-9669) were eligible to enroll and be treated with LDV/SOF for 12 weeks. HCV RNA was measured using the Abbott assay with a lower level of quantitation (LLOQ) of 12 IU/ml. The primary endpoint was defined as HCV RNA viral load (VL) below the LLOQ 12 weeks after completion of therapy (SVR12). Deep sequencing of NS5B, NS3 and NS5A regions were performed at two time points (prior to initial therapy and at the time of relapse, prior to initiation of retreatment) by Illumina next generation sequencing technology.

Results: The study enrolled 34 participants, and 31 (91%) have completed therapy with 12 weeks of LDV/SOF. One patient remains on therapy after a delayed start, one patient was lost to follow up after Day 0 and another withdrew consent at week 4. Participants were predominantly male (82%) and black (85%), with a median age of 60.5 years (IQR 57.0–63.8) and BMI of 26.8 kg/m^2 (IQR 25.3–29.3). Baseline HCV VL was 1.3×10^6 IU/mL (IQR 5.8×10^5–3.9×10^6), 73.5% (25/34) were infected with HCV genotype 1a, and median Metavir fibrosis stage was 1. Median time from relapse to retreatment was

22 weeks (IQR 18–23). We report SVR4 rates of 94% (31/33; ITT) and 100% (31/31, per protocol). For SVR rates by original treatment group in a per protocol analysis, see Figure. Prior to initial therapy, 3 patients had variants consistent with high levels of resistance (>5 fold) in NS3 and 8 in NS5A, and all but one patient lost to follow up went on to achieve SVR4. Data collection is ongoing, and SVR12 data will be presented at the conference.



Conclusions: For the first time, we demonstrate a high SVR rate following retreatment with DAAs in patients who have previously failed DAA-only therapy.

LP10

EFFICACY AND SAFETY OF VITAMIN E FOR NONALCOHOLIC STEATOHEPATITIS: COMBINED ANALYSIS OF THREE CONTROLLED TRIALS

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Introduction: Vitamin E (d-alpha-tocopherol) has been part of the treatment for nonalcoholic steatohepatitis (NASH) in three NIDDK-sponsored clinical trials as part of the NASH Clinical Research Network: PIVENS compared vitamin E to pioglitazone and placebo in non-diabetic adults, TONIC compared vitamin E to metformin and placebo in non-diabetic children; FLINT compared obeticholic acid to placebo in diabetic and non-diabetic adults, and 23% of placebo-treated patients were taking vitamin E. We report the combined efficacy and safety of vitamin E in 347 patients; we also compare effect sizes of vitamin E and the other NASH treatments across the three studies.

Material and Methods: Histologic improvement, defined as ≥2 point improvement in NAS with no worsening of fibrosis, and NASH resolution measured effectiveness. Safety measures included incidence of cardiac events and changes in lipid levels. Logistic regression models were used to summarize the odds ratio (OR) effects, confidence limits, and p-values on the combined effect.

Results: NASH patients (n = 155) treated with vitamin E vs. those not treated with vitamin E (n = 192) were more likely to achieve histologic improvement [45% vs 22%, OR = 2.9, 95% CI 1.8, 4.7; p < 0.0001], with an effect size comparable to pioglitazone, metformin, and obeticholic acid (ORs 4.1, 2.7, 3.1, respectively). Vitamin E treated patients also had higher rates of NASH resolution [38% vs 20%, OR = 2.4, 95% CI 1.5, 3.9; p < 0.001], with comparable effect sizes to pioglitazone, metformin, and obeticholic acid (ORs 3.4, 1.8, 1.8, respectively). Incidence of cardiac events was not significantly different in vitamin E vs. no vitamin E groups (6.9% vs. 7.6%, p = 0.85). There were no significant differences in net changes in total cholesterol, HDL cholesterol, LDL cholesterol or triglycerides.

Table 1. Effects of four anti-NASH treatments on histologic improvement and resolution of NASH

Group	%	x/N	OR	95% CI	P
Histologic improvement [a]					
Vitamin E	45%	70/155	2.9	1.8, 4.7	<0.0001
No vitamin E (Placebo)	22%	42/192	1.0		
Pioglitazone	56%	39/70	4.1	2.0, 8.4	0.0001
Placebo	24%	17/72	1.0		
Metformin	48%	24/50	2.7	1.1, 6.4	0.02
Placebo	26%	12/47	1.0		
Obeticholic acid	49%	50/102	3.1	1.7, 5.8	0.0002
Placebo	23%	23/98	1.0		
Resolution of NASH [b]					
Vitamin E	38%	56/148	2.4	1.5, 3.9	<0.001
No vitamin E (Placebo)	20%	37/184	1.0		
Pioglitazone	47%	33/70	3.4	1.6, 7.1	0.001
Placebo	21%	15/72	1.0		
Metformin	41%	16/39	1.8	0.7, 4.6	0.24
Placebo	28%	11/39	1.0		
Obeticholic acid	22%	22/102	1.8	0.8, 3.8	0.13
Placebo	13%	13/98	1.0		

Vitamin E data from PIVENS and TONIC vitamin E treatment groups, and FLINT placebo group patients reporting vitamin E use at baseline. Pioglitazone data from PIVENS; metformin data from TON1C, and obeticholic acid data from FLINT.
[a] Histologic improvement as defined in the FLINT Trial protocol, defined as a decrease of 2 or more points in the NAS and no worsening in the fibrosis score.
[b] Resolution of NASH defined as no NASH at end of treatment biopsy among patients with borderline or definite NASH at baseline.

Conclusions: Vitamin E treatment was associated with significant improvement in NASH histology and resolution of NASH in a pooled analysis of three trials of adult and pediatric NASH. The magnitude of the effect of vitamin E on NASH was comparable to pioglitazone, metformin, and obeticholic acid. No increase in cardiovascular events or adverse lipid profiles was observed with vitamin E treatment. These data support the use of vitamin E as a treatment for NASH.

LP11

WARFARIN ANTICOAGULATION FOR LIVER FIBROSIS IN PATIENTS TRANSPLANTED FOR HEPATITIS C (WAFT-C): RESULTS AT ONE YEAR

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Introduction: Apart from treating the underlying aetiology no therapy is currently licensed for the prevention or treatment of liver fibrosis. In patients transplanted for hepatitis C (HCV) 30% develop rapid fibrosis with progression to cirrhosis within 5 years. FXa and thrombin promote hepatic fibrosis by stellate cell activation and there is an association between pro-thrombotic tendencies

and accelerated fibrosis in HCV pre-transplantation. Direct FXa inhibition and warfarin anticoagulation both ameliorate hepatic fibrosis in animal models. There is now a need to evaluate the effect of anticoagulation on hepatic fibrosis in human studies.

Aims: To evaluate the safety and impact of warfarin anticoagulation on liver fibrosis progression in patients transplanted for HCV infection.

Material and Methods: WAFT-C is a prospective multi-centred, randomised open-label controlled trial designed to investigate the impact of anticoagulation on fibrosis progression over one and two years post liver transplantation. Patients are randomised by centre and gender to standard post transplant care (control group) or standard of care with warfarin anticoagulation maintaining an INR between 2–3 (warfarin group). Interim intention to treat and per protocol analyses were undertaken for fibrosis scores at year one post transplantation. The primary endpoint was the proportion of patients with an increase of ≥2 in their Ishak fibrosis score by per protocol analysis.

Results: 76 patients have been randomised (control group: n = 39; mean age 52.3 yrs; M:F=33:6. warfarin group: n = 37; mean age 53.2 yrs; M:F=30:7). Intentional to treat analysis of year one fibrosis scores (n = 65) indicates a non-significant reduction in the proportion of patients with an increase in fibrosis score between the control and warfarin groups (26% vs 13%, p > 0.05). Per protocol analysis (n = 53) after exclusion of biliary disorders (control group n = 2, warfarin group n = 3) and non compliant patients (failed to start anticoagulation, n = 5; <8 weeks of anticoagulation, n = 2) demonstrated a significant reduction in the proportion of patients with an increase in fibrosis score between the control group and warfarin group at year one (23.3% vs 0%, p = 0.01). No patients were withdrawn due to severe adverse events directly secondary to anticoagulation.

Conclusions: Results of the one year endpoint suggest that warfarin anticoagulation may benefit in reducing HCV related fibrosis one year post transplantation. Two year endpoint data is required to validate these findings.

LP12

SILYMARIN FOR THE TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS: INTERIM ANALYSIS OF A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Introduction: Silymarin, derived from the milk thistle plant, *Silybum marianum*, has been used as a herbal remedy for diseases of the liver. Its anti-oxidant, anti-inflammatory and anti-fibrotic properties have been demonstrated in various in vitro and animal models, and may be useful for the treatment of non-alcoholic steatohepatitis (NASH).

Material and Methods: This is a randomized, double-blind, placebo-controlled study of silymarin 700 mg t.i.d. for the treatment of NASH. All included patients had biopsy-proven NASH, were given lifestyle advice, and received either silymarin or placebo for 48 weeks. A repeat liver biopsy was performed at the end of the study. Histology was reported using the NASH Clinical Research Network scoring system.

Results: A total of 64 patients completed the study at the time of this interim analysis. Mean age was 50.2±11.4 years and consisted of 43.8% males. The baseline characteristics were comparable between the silymarin (n = 30) and placebo (n = 34) groups. The primary endpoint of the study (defined as at least 30% improvement in the non-alcoholic fatty liver disease activity score, NAS) was met in a higher percentage of patients in the silymarin group compared to the placebo group but this was not statistically significant (33.3% vs. 20.6%, p = 0.517). Patients in both the silymarin and placebo groups experienced significant improvement in NAS ($\Delta = -0.733$, p = 0.003 in the silymarin group; $\Delta = -0.706$, p = 0.006 in the placebo group). The percentage of patients with improvement in NAS was not significantly different between the silymarin and placebo groups (60.0% vs. 58.5%, p = 0.924). However, significantly more patients in the silymarin group experienced NASH resolution (defined as NAS <3) compared to the placebo group (13.3% vs. 0%, p = 0.043). There was also a significant decrease in the fibrosis stage in the silymarin group ($\Delta = -0.367$, p = 0.019). This was not observed in the placebo group ($\Delta = +0.147$, p = 0.282). A significantly higher percentage of patients in the silymarin group had improvement in fibrosis stage compared to the placebo group (36.7% vs. 14.7%, p = 0.043). In addition, four patients in the placebo group developed cirrhosis while none of the patients in the silymarin group did.

Conclusions: A significantly higher percentage of patients experienced NASH resolution and improvement in fibrosis stage after 48 weeks of treatment with silymarin compared to placebo.

LP13

EFFECT OF LONG TERM VIRAL SUPPRESSION WITH SOFOSBUVIR + RIBAVIRIN ON HEPATIC VENOUS PRESSURE GRADIENT IN HCV-INFECTED PATIENTS WITH CIRRHOSIS AND PORTAL HYPERTENSION

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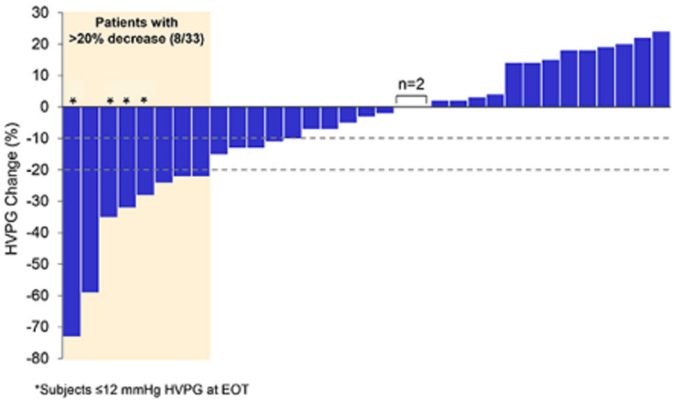
Introduction: Portal hypertension is a major predictor of clinical outcomes and anti-viral response in HCV cirrhosis. The effect of viral suppression and SVR on HVPG has not been characterized in patients treated with DAA's.

Material and Methods: FFifty CPT A (n = 18) and B (n = 32) cirrhotic patients with portal hypertension (HVPG >6 mmHg), were randomized to receive 48 weeks of open-label sofosbuvir 400 mg and ribavirin at Day 1 or after a 24 week observation period. The primary endpoint was SVR12. Secondary endpoints included changes in HVPG, laboratory parameter values, MELD and CPT scores.

Results: Patients were predominantly male (76%), Caucasian (90%), prior treatment failures (80%), genotype 1 and 3 (88%) with mean baseline HCV RNA 6.1 $\log_{10}$ IU/mL. In the observation arm, 4 patients discontinued and never received treatment. All patients who completed treatment had viral suppression to undetectable by week 8 on treatment and SVR12 was achieved in 72% (33/46) of patients (2 on-treatment failures and 11 relapses). At study entry mean baseline HVPG was 16.4 mmHg in the observation group and 17.5 mmHg in the treatment group. Over the 24 week observation period the median change in HVPG was 0 mmHg (−7.0 to +4.5 mmHg). Of the 37 patients who had paired baseline and end of treatment (EOT) HVPG measurements there was a median change of −0.5 mmHg. Of the 33/37 (89%) patients with HVPG ≥12 mmHg at baseline, a ≥20% decrease from baseline was observed in 8/33 (24%) patients at the EOT and 4 patients reduced their pressure to less than 12 mmHg (see Figure). Improvements in MELD score from baseline to follow-up week 4 were seen in 37% (15/41) of patients with an average decline of −1.6. Improvement in CPT score was seen in 69% (18/26) of CTP B patients. In an

univariate analysis, a higher baseline MELD score (≥10) was the only factor significantly associated with a ≥20% decrease in HVPG (p = 0.04). 5 of 8 patients who had a ≥20% decrease in HVPG had improvements in MELD (mean decrease, −1.5) and all 8 patients had improvements in albumin (mean increase, +0.50 g/dL).



Conclusions: SOF+RBV for 48 weeks in patients with HCV and portal hypertension was well tolerated with an SVR rate of 72%. Improvement in MELD and CPT were common during and at end of treatment. HVPG at EOT decreased significantly in patients with higher MELD scores, although the full effects of SVR and viral eradication on HVPG may manifest later and is being explored in these patients at 1 year post treatment.

LP14

A PHASE 3, RANDOMISED, OPEN-LABEL STUDY TO EVALUATE THE EFFICACY AND SAFETY OF 8 AND 12 WEEKS OF SIMEPREVIR (SMV) PLUS SOFOSBUVIR (SOF) IN TREATMENT-NAÏVE AND -EXPERIENCED PATIENTS WITH CHRONIC HCV GENOTYPE 1 INFECTION WITHOUT CIRRHOSIS: OPTIMIST-1

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Introduction: In a Phase II study (COSMOS), an all oral, once-daily (QD) combination of SMV (Hepatitis C virus [HCV] NS3/4A protease inhibitor) + SOF (HCV nucleotide-analogue NS5B polymerase inhibitor) +/− ribavirin for 12 or 24 weeks (wks) achieved high sustained virologic response (SVR) rates and was well tolerated in treatment-naïve and prior null responder patients (pts), including METAVIR F3–F4 pts. This Phase 3, randomised, open-label study (OPTIMIST-1; NCT02114177) evaluated efficacy and safety of 12 and 8 wks of SMV+SOF, in treatment-naïve or -experienced HCV genotype (GT)1-infected pts without cirrhosis.

Material and Methods: Randomisation (1:1; stratified by HCV GT subtype ±Q80K, *IL28B* GT, treatment history) to 12 or 8 wks of SMV 150 mg QD + SOF 400 mg QD. Superiority of SMV+SOF for 12 and 8 wks, each vs a historical control (HC; derived from published data), was assessed. If both were superior, non-inferiority of 8 vs 12 wks of SMV+SOF was to be assessed. Primary efficacy endpoint was SVR 12 wks after end of treatment (EOT; SVR12). Safety and pt-reported outcomes were assessed.

Results: A total of 310 pts received treatment (male, 55%; median age, 56 years; Black/African American, 18%; *IL28B* CC, 27%; GT1a/1b 75/25%; treatment-naïve [n = 218, 70%]; treatment-experienced [n = 92, 30%]). SVR12 with 12 wks of SMV+SOF (97% 95% confidence interval [CI] 94–100%) was superior to HC (87%). SVR12 with 8 wks of SMV+SOF (83% 95% CI 76–89%]) did not achieve superiority vs HC (83%). Other endpoints are summarised in Table 1.

Adverse events (AEs) occurred in 103 (67%) and 97 (63%) pts receiving 12- or 8-wk treatment, respectively, and were mainly Grade 1/2 (64% and 61%). Most frequent AEs: headache, fatigue and nausea (12-wk arm: 14%, 12% and 15%; 8-wk arm: 17%, 15% and 9%, respectively). AEs of interest, increased bilirubin and rash, occurred in 1 (1%) and 10 (7%) pts in the 12-wk arm vs 1 (1%) and 12 (8%) pts in the 8-wk arm, respectively. No pts discontinued treatment due to an AE. Serious AEs were infrequent (12-wk arm, 1 pt [1%]; 8-wk arm, 3 pts [2%]). No deaths occurred. Patient-reported symptoms and quality of life significantly improved from baseline to SVR12 in both treatment arms (Table 2). Additional data will be presented.

Table 1. SVR12 (overall and sub-groups) with SMV 150 mg QD + SOF 400 mg QD

	n/N (%)	
	12-week arm	8-week arm
SVR12 [a]	150/155 (97)	128/155 (83)
Treatment-naïve	112/115 (97)	88/103 (85)
Treatment-experienced	38/40 (95)	40/52 (77)
HCV GT1a	112/116 (97)	92/116 (79)
HCV GT1b	38/39 (97)	36/39 (92)
IL28B CC	43/43 (100)	38/41 (93)
IL28B non-CC	107/112 (96)	90/114 (79)
On-treatment failure [b]	0	0
Viral relapse [c]	4/154 (3)	27/155 (17)

[a] HCV RNA <25 IU/mL detectable/undetectable 12 weeks after end of treatment.
[b] HCV RNA <25 IU/mL detectable or ≥25 IU/mL at end of treatment.
[c] HCV RNA <25 IU/mL undetectable at end of treatment and ≥25 IU/mL during follow up.

Table 2. Patient-reported outcomes

Patient-reported outcome	Mean (SD) change from baseline			
	12-week arm		8-week arm	
	SVR12	No SVR12	SVR12	No SVR12
HCV-SIQv4 OBSS [a]	−3.9 (0.96)	6.7 (7.07)	−2.9 (0.87)	3.4 (2.82)
FSS [b]	−0.5 (0.15)	1.4 (0.99)	−0.6 (0.12)	−0.0 (0.22)
CES-D [c]	−0.2 (0.73)	5.3 (5.33)	−2.5 (0.61)	4.2 (1.94)
EQ-5D VAS [d]	4.1 (1.40)	−7.0 (7.51)	6.5 (1.39)	−0.6 (2.63)

[a] HCV-SIQv4 OBSS, Hepatitis C Symptom & Impact Questionnaire version 4 Overall Body System Score (range 0 [no symptoms] to 100 [maximum severity for all 29 symptoms]; mean reduction of ≥5 points is clinically important).
[b] FSS, Fatigue Severity Scale (range 1 [no fatigue] to 7 [severe fatigue]; mean reduction of 0.6 points is clinically important).
[c] CES-D, Center for Epidemiologic Studies Depression Scale (range 0 [no depressive symptoms] to 60 [severe depression on 20 symptoms]; mean reduction of ≥3 points is clinically important).
[d] EQ-5D VAS, EuroQol-5 Dimension questionnaire Visual Analogue Scale (range 0 [worst possible health] to 100 [perfect health]; mean increase of ≥5 points is clinically important).

Conclusions: SMV+SOF for 12 wks was superior to HC, and SMV+SOF for 8 wks did not achieve superiority vs HC in treatment-naïve and -experienced HCV GT1-infected pts without cirrhosis. SMV+SOF was well tolerated.

Funded by Janssen

LP15

SEPARATE AND COMBINED EFFECTS OF OBETICHOLIC ACID AND WEIGHT LOSS IN NONALCOHOLIC STEATOHEPATITIS (NASH)

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Introduction: Obeticholic acid (OCA) is a potent farnesoid-X nuclear receptor agonist. In a 72-week, randomized controlled trial (FLINT) in 283 patients with NASH, OCA was found to be superior to placebo in improving ALT levels, liver histology and resulted in more weight loss (−2.3 kg) than placebo (0.0 kg) (Lancet, 2014). We assessed the separate and combined effects of weight loss and OCA.
Material and Methods: This secondary analysis was limited to the 200 patients with a pre- and end-of-treatment liver biopsy. Liver histology was scored using a standardized system for disease activity (NAS) and fibrosis. Weight loss was defined as ≥ −2 kg decline by end of treatment.
Results: Weight loss occurred in 42% of OCA vs 30% of placebo patients with paired biopsies ($p = 0.08$). Mean change in NAS was greater in OCA patients who lost vs. did not lose weight (−2.5 vs −1.2, $p < 0.001$), an effect seen to a lesser extent with placebo (−1.4 vs −0.4, $p = 0.008$). Fibrosis scores improved in 49% of OCA patients who lost weight vs 25% of those who did not ($p = 0.02$), but fibrosis improvement with placebo was similar with and without weight loss (17% vs 20%). ALT levels decreased more in patients who lost vs. did not lose weight in both OCA (−42 vs −33 U/L) and placebo (−40 vs −7 U/L). In contrast, LDL cholesterol levels rose (+23 mg/dL) in OCA patients who lost weight, but fell in placebo with weight loss (−17 mg/dL) and did not change without weight loss in either OCA or placebo (0 and −2 mg/dL). HDL cholesterol decreased (−0.6 mg/dL) in OCA patients who lost weight, but increased in placebo with weight loss (+3.6 mg/dL).
Conclusions: OCA led to weight loss in 42% of patients. Both OCA therapy and weight loss had beneficial effects on NAFLD severity. Improvements in fibrosis was greatest in OCA patients with weight loss. Paradoxically, weight loss during OCA treatment was associated with potentially adverse changes in the lipid profile.

LP16

MUTATIONS IN DCDC2, ENCODING DOUBLECORTIN DOMAIN-CONTAINING PROTEIN 2, CAUSE NEONATAL SCLEROSING CHOLANGITIS

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Introduction: Cholangiopathies of neonatal onset are typified by biliary atresia (BA), with inflammatory obliteration of bile-duct lumina near the porta hepatis. A similar process without complete occlusion is called neonatal sclerosing cholangitis (NSC). BA and NSC are very alike histopathologically at biopsy of peripheral liver. NSC, unlike BA, is greatly enriched in consanguineous kindreds, strongly suggesting autosomal recessive inheritance. The cause of NSC is not known.
Material and Methods: With ethics-committee approval, archival samples from 13 children with NSC (7 male), 4 from consanguineous families, were selected for whole exome sequencing. The data were analysed using several open source and commercial software packages. Variants were filtered for homozygosity, pathogenicity, minor allele frequency (MAF), and expression pattern of the encoded proteins.
Results: Sequencing data were obtained in all cases. Variants present with MAF >1% in the 1000 Genome Project data, and variants flagged as low quality (quality score <30, long homopolymer runs >5, depth <5) were excluded. Three patients were homozygous for mutations in *DCDC2*, encoding doublecortin domain containing 2 (DCDC2), a ciliary protein expressed in small-duct cholangiocytes, with another 3 patients showing compound heterozygous mutations in *DCDC2*. An additional sibling had the same *DCDC2* mutations. All mutations were confirmed on Sanger sequencing. All mutations were protein truncating. In the 6 patients whose liver tissue was available, immunostaining with monoclonal antibody against human DCDC2 showed no expression. In material suitable for transmission electron microscopy (5 patients, including the siblings), cholangiocytes lacked primary cilia. All patients but one, who developed end-stage renal disease of unknown aetiology, had normal renal function; the sibling pair each had a single small renal cyst. One patient died awaiting liver transplantation (LT). Of 5 who came to LT, one died 2 years later; 4 are well.
Conclusions: DCDC2 is known to be important in ciliary regulation. Among 14 patients with NSC, 7 had mutations in *DCDC2* (6 of 13 families). Our data indicate that mutations in *DCDC2* underlie a substantial proportion of NSC. Histopathologic correlation suggests that NSC is mediated by ciliary abnormalities.

LP17

NOVEL APPROACH FOR THE PREVENTION OF RECURRENT HEPATITIS C IN LIVER TRANSPLANT RECIPIENTS: PRELIMINARY RESULTS FROM ONGOING PHASE III TRIAL WITH CIVACIR®

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Introduction: HCV remains the leading cause for liver transplantation (LT) and recurrent HCV disease the most frequent cause of graft loss. Although direct acting antivirals have the potential to reduce HCV RNA levels prior to LT, post-LT recurrence occurs in 30%>50% in patients receiving sofosbuvir/ribavirin pre-LT. Prevention of reinfection independent of genotype and severity

of cirrhosis is highly desirable because it simplifies post-LT management.

Material and Methods: In this adaptive open-label, prospective randomized phase III North American clinical trial (Study 988, NCT01804829) conducted in 2 parts, any antiviral therapy (AVT) pre-LT (resulting in RNA <100 IU/ml prior to transplant) is followed in the active treatment arms by Hepatitis C Immune Globulin (HCIG, Civacir®) peri- and post-LT to prevent HCV infection after LT. In part I (dose finding), patients were randomized 1:1:1 to active (200 or 300 mg/kg) or control arms. Active group patients received 16 infusions of Civacir in the peri- and immediate post-LT period for 10 weeks. Control patients received standard of care (no treatment) immediately after LT. The primary endpoint is sustained virologic response SVR12.

Results: Three quarters (N = 63 as of 27 Jan 2015) of 84 planned patients have been enrolled. Preliminary efficacy evaluations justified continued enrollment in the 300 mg/kg arm but not in the 200 mg/kg low dose arm (closed by amendment). Treatment and control arms are comparable at baseline. Mean age of patients is 61 and 84% are male. 93% of patients were treated with sofosbuvir-based regimens pre-LT, for a median duration of 63.5 days including 9.4% with treatment <4 weeks. To date, 1/21 (5%) reinfection was observed in the 300 mg/kg arm. In the control and 200 mg/kg arm 7/22 (32%) and 6/20 (30%) reinfections occurred, respectively. At day 6, 14, 21 and 28 post-LT the median anti-HCV antibody titer was 440, 312, 274 and 215 U/ml in the 300 mg/kg and 39, 44, 53 and 63 U/ml in the control arm. Civacir has been well tolerated with no drug-related serious adverse events to date. The most frequent reported AEs were those well-known for human immunoglobulins, underlying disease, and surgical associated events.

Table 1. Status of patient recruitment and pre-LT antiviral treatment

Dose group	# pts	Pre-LT sofosbuvir treatment		Reinfections	
		%[a]	Median days[b]	Number	%
Control	22	90%	66	7	32%
200 mg/kg	20	90%	61	6	30% (p = 1.00)
300 mg/kg	21	94%	60	1	5% (p = 0.046)

[a] % of patients recerving sofosbuvir based therapy pre-LT.
[b] Median days of sofosbuvir treatment pre-LT.

Conclusions: Patients with HCV receiving AVT prior to LT are still at risk to reinfect. In this phase III clinical study HCIG therapy is used as a prophylactic approach immediately after LT. Preliminary data suggest that Civacir can prevent HCV-reinfection in patients on pre-LT AVT.

LP18

OBETICHOLIC ACID FOR NASH: BENEFITS IN A HIGH RISK SUBGROUP AND THE EFFECTS OF CONCOMITANT STATIN USE

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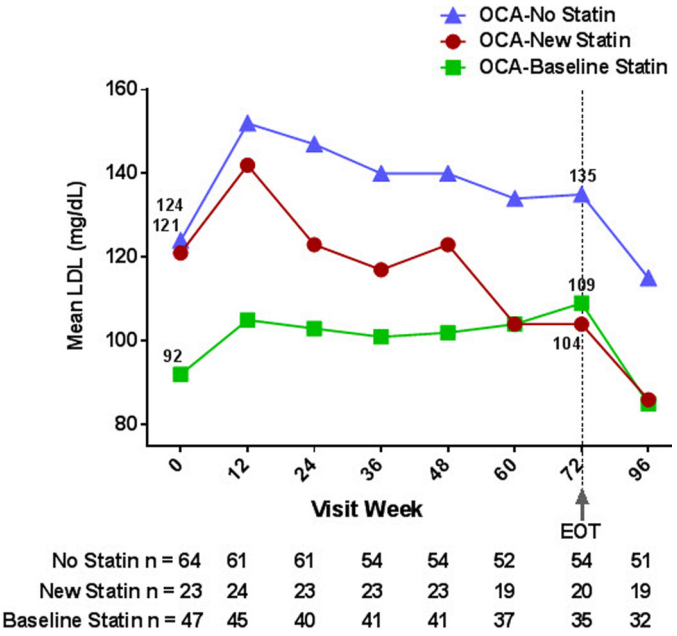
Introduction: The Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) Trial was a 72 week placebo-controlled, double-blind trial in patients (N = 283) with non-cirrhotic, nonalcoholic steatohepatitis (NASH). Obeticholic acid (OCA) improved liver histology including fibrosis but was associated with pruritus and elevations of serum total and LDL cholesterol (Neuschwander-Tetri, Lancet 2014). The aims of these secondary analyses were to determine the effect of OCA versus Placebo in the subgroup of patients with more severe NASH and to assess the effects of concomitant statin use on serum LDL cholesterol levels.

Material and Methods: The subgroup of patients at higher risk for liver-related outcomes was defined as those with stage 2–3 fibrosis or stage 1 fibrosis with diabetes, obesity or ALT ≥60 (associated with fibrosis progression) (OCA N = 85; Placebo N = 77). The effect of statin use on changes in LDL cholesterol was assessed in the entire cohort based on patient-reported statin use before and during treatment, initiation during treatment, or no statin use before or during treatment.

Results: Significant histologic improvements were observed in the OCA vs Placebo high-risk subgroup: NAS improved by ≥2 points in 50% OCA vs 31% Placebo (p = 0.001), NASH resolution occurred in 18% OCA vs 6.5% Placebo (p = 0.03), and fibrosis regressed by at least one stage in 39% OCA vs 22% Placebo (p = 0.012). In addition, fibrosis progression with OCA was attenuated (16% OCA vs 29% Placebo [p = 0.047]). The histologic improvement in OCA-treated patients occurred regardless of fibrosis stage at baseline. LDL cholesterol increased during OCA treatment in patients on statins at baseline, but levels did not exceed those of Placebo-treated patients not on statins. Statin initiation during OCA treatment reversed LDL to below pre-OCA baseline levels.



Conclusions: In the subgroup of FLINT patients at higher risk of progression to cirrhosis, OCA treatment improved the features of NASH, NASH resolution, fibrosis regression and decreased fibrosis progression. The OCA-related LDL increase appeared to be reversed by initiating statin therapy during OCA treatment. The clinical significance of these OCA-related treatment effects remains to be determined and warrants further study.

LP19
EARLY INTRODUCTION OF SUBCUTANEOUS (S.C.) HEPATITIS B IMMUNOGLOBULIN (HBIg) PROVIDES EFFECTIVE PROPHYLAXIS FOR HEPATITIS B VIRUS (HBV) REINFECTION AFTER LIVER TRANSPLANTATION (TX)

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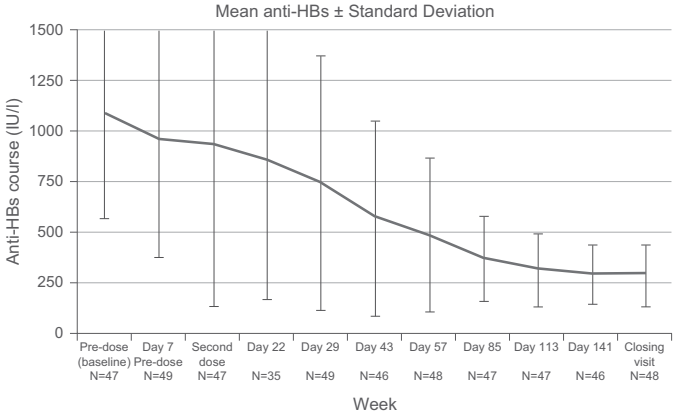
Introduction: Subcutaneous HBIg (Zutectra®) in maintenance liver transplant patients assures hepatitis B surface antibody (anti-HBs) serum trough level >100 IU/l, the minimum threshold for effective HBV reinfection prophylaxis. Data regarding early s.c. administration of HBIg are lacking.
Material and Methods: In a prospective, open-label, single-arm study, HBV DNA-negative patients undergoing liver tx for HBV infection were switched at 8–18 days post-tx from i.v. to s.c. HBIg (500 or 1,000 IU once a week or once every 2 weeks) if they were HBsAg negative at time of switch, and were monitored to month 6 post-tx. All patients received concomitant anti-HBV nucleoside therapy. Self-injection (or by a caregiver) could be started after week 4 if anti-HBs trough level was >100 IU/l and if patients complied with the injection technique. Primary endpoint was failure rate during 6 months, defined as serum anti-HBs ≤100 IU/l or HBV reinfection with serum anti-HBs >100 IU/l.
Results: 49 patients were recruited (20 dosed once a week, 29 dosed once every 2 weeks), of whom 47 (95.9%) continued treatment until month 6. By week 14, 47 patients were self-administering (n = 35) or being injected by a caregiver (n = 12). No treatment failures occurred during the 6-month study treatment period i.e. all patients maintained serum HBs antibody concentrations ≥100 IU/L and remained HBsAg negative. All patients tested for HBV DNA remained negative (45/45). Mean anti-HBs declined successively to month 6 to a protective titer of 250 IU/l (figure). No clinical symptoms consistent with HBV reinfection were observed. Only one non-serious adverse event (mild injection site hematoma) was assessed as treatment-related. No serious drug-related adverse events occurred. All 44 patients who completed an end-of-study questionnaire reported that s.c. injection was convenient and were satisfied with s.c. HBIg.



Conclusions: Early switch to s.c. HBIg (between days 8–18) after liver tx maintained serum anti-HBs at a level which effectively prevented HBV reinfection in all patients. Treatment was well-tolerated. Self-administration of s.c. HBIg as part of combination treatment with HBV virostatic therapy appears a successful and convenient strategy for preventing HBV reinfection.

LP20
SAFETY AND EFFICACY OF ALL-ORAL DAA REGIMENS IN HIV–HCV COINFECTED CIRRHOTIC PATIENTS FROM THE PROSPECTIVE ANRS CO13 – HEPAVIH COHORT

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Introduction: Cirrhotic HIV–HCV coinfected patients have long been considered as difficult-to-treat patients with a high mortality rate. DAA regimens have changed this paradigm in HCV-monoinfected patients. Real-life efficacy and tolerance data with sofosbuvir (SOF)-based combinations in cirrhotic HIV-coinfected patients are scarce and urgently needed.
Material and Methods: Cirrhotic HIV–HCV coinfected patients prospectively enrolled in the CO13 HEPAVIH cohort sponsored and funded by ANRS, and initiating an all-oral DAA regimen were included. Safety and virologic responses (VR: HCV-RNA <15 UI/mL) at week 4 (W4) and end of treatment (EOT) as well as sustained virological response at 4 (SVR4) and 12 (SVR12) weeks were assessed.
Results: 106 patients initiated SOF-based regimen between 01/2014 and 01/2015 (median age: 54; 80% male; CDC A/B/C: 30/36/34%;

Child–Pugh A/B/C: 87/10/3%; 86% with HIV-RNA <50 copies/mL; 104 on continuous antiretroviral treatment (cART); median CD4 at baseline: 462/mm^3 [IQR: 265–628]). History of previous HCV treatment was: naïve 28%, treatment failure with PegIFN+Ribavirin (PR) 57%, treatment failure with PR+first generation protease inhibitor 11%, treatment failure with PR+others 4%. HCV genotype (Gt) were 1a (32%), 1b (12%), 1 other (10%), 2 (4%), 3 (19%) and 4 (23%). Four regimens were used: SOF+R in 22 patients (Gt1 28%; Gt2 18%; Gt3 36%; Gt4 18%), SOF+daclatasvir±R in 75 (Gt1 64%; Gt3 15%; Gt4 21%), SOF+ledipasvir in 3 (2 Gt1 and 1 Gt3) and SOF+simeprevir in 6 (2 Gt1 and 4 Gt4). On February 24th, 2015, 100%, 77%, 69% and 42% of the patients reached W4, EOT, and W4 and W12 post-treatment, respectively. No premature stop of DAA was reported in relation with tolerance or efficiency. Adverse events were reported in 22 patients (digestive 5%, anemia 36%, asthenia 14%, others 45%), and R was stopped in 3 patients and dose-adjusted in 9. During treatment and post-treatment period, clinical events in relation with cirrhosis were reported in 8% (hepatocellular carcinoma in 2 and decompensation in 5) and with HIV in 4% of the patients. VR was observed in 52% of the patients at W4, 100% at EOT. SVR4 and SVR12 were 100% and 93% for the patients reaching this endpoint, respectively.

Conclusions: In this prospective real-life cohort, new all-oral DAA regimens were well tolerated and associated with very encouraging virological efficacy in cirrhotic HIV–HCV coinfected patients. This should not alleviate the surveillance for liver-related events in these patients.

LP21

THE MACROPHAGE ACTIVATION MARKERS SOLUBLE CD206 AND CD163 PREDICT MORTALITY IN PATIENTS WITH LIVER CIRRHOSIS AND ACUTE-ON-CHRONIC LIVER FAILURE (ACLF)

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Introduction: Activation of Kupffer cells, constituting >80% of the entire body macrophage population, play a key role in inflammation and may be involved in the development and course of ACLF. We explore this hypothesis by measuring the specific macrophage activation markers sCD163 and sCD206 in plasma and their relationship to short- (1–3 months) and long-term mortality (6 months) in 853 cirrhotic patients from the CANONIC study.

Material and Methods: 86 patients had no ascites and no ACLF, 580 had ascites but no ACLF and 100, 66 and 19 had ACLF-grade I (ACLF-1), ACLF-II, and ACLF-III, respectively. We used in house ELISA for measurement of sCD163 and sCD206

Results: We found a stepwise increase ($p < 0.001$) in sCD163 (7.1±4.5; 9.2±5.6; 11.8±8.2; 16.9±10.3; 24.4±11.3 mg/l, respectively) and sCD206 (0.67±0.4; 0.91±0.5; 0.96±0.5; 1.27±0.5; 1.64±0.8 mg/l) with increasing disease severity. In patients with and without ACLF both sCD206 and sCD163 were independently associated with short and long-term mortality and showed equal or higher predictive accuracy than MELD and CLIF-C Acute Decompensation (AD) and CLIF-C ACLF scores (s). For patients without ACLF, addition of sCD206 to MELDs (data not shown) and CLIF-C-ADs significantly improved their prognostic performance for long-term mortality. For patients with ACLF, addition of sCD163 to MELDs and CLIF-C-ACLFs improved their prognostic performance for short-term mortality (Table 1).

Conclusions: The graded increase in sCD163 and sCD206 and their close association with mortality suggest the importance of liver resident macrophages in the development and course of ACLF. Accordingly, the inclusion of the macrophage biomarkers sCD206 to the CLIF-C-AD and sCD163 to the CLIF-C-ACLF scores improved the prognogstic predictive performance beyond that of the original scores.

LP22

PHASE 1 RESULTS FROM PXS-4728A, A SELECTIVE SSAO/VAP-1 INHIBITOR, FOR THE TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS

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Introduction: SSAO/VAP-1 is an ectoenzyme located on endothelial cells, adipocytes and smooth muscle cells that facilitates the migration of leukocytes and lymphocytes to the site of inflammation whilst also inducing oxidative stress through metabolism of primary amines into aldehydes, hydrogen peroxide and ammonia. Plasma SSAO/VAP-1 is shed from the hepatic endothelium into the circulation, and its concentration is positively correlated with liver fibrosis and cirrhosis. Recently, compelling evidence has been provided that the enzymatic activity of SSAO/VAP-1 is involved in the development of fatty liver diseas (Weston et al., J Clin Invest. 2015; 125(2): 501–520. doi: 10.1172/JCI73722).

Results: PXS-4728A is a very selective, mechanism-based small molecule inhibitor of SSAO/VAP-1 with nanomolar potency, good oral bioavailability and desirable developability properties. PXS-4728A reduces infection and lipopolysaccharide, rhinovirus or

Table 1 (abstract LP21). Receiver operating characteristic area under the curve (AUC) for CLIF-Consortium Acute Decompensation (CLIFC-AD) score and CLIF-ACLF score alone or combined with sCD163 and sCD206 for the prediction of 28, 90 and 180 days mortality

	C-index (95% CI)			
	No ACLF patients (n = 543)		ACLF patients (n = 153)	
	90-days mortality	180-days mortality	28-days mortality	90-days mortality
sCD163 (mg/L) [log-transf.]	0.69 (0.63, 0.76)	0.68 (0.63, 0.73)		
sCD206 (mg/L) [log-transf.]	0.76 (0.71, 0.81)	0.74 (0.69, 0.78)		
CLIFC-AD score	0.74 (0.68, 0.79)	0.72 (0.68, 0.77)		
+ Log-sCD163	0.78 (0.73, 0.83)	0.76 (0.72, 0.80)		
+ Log-sCD206	0.80 (0.76, 0.85)	0.78 (0.74, 0.82)		
Comparison C-index CLIFC-AD vs CLIFC-AD + 206	**0.028**	**0.024**		
sCD163 (mg/L) [log-transf.]	Not applicable		0.71 (0.63, 0.79)	0.66 (0.59, 0.73)
sCD206 (mg/L) [log-transf.]			0.67 (0.59, 0.75)	0.66 (0.59, 0.72)
CLIFC-ACLF score			0.75 (0.68, 0.83)	0.71 (0.64, 0.77)
+ Log-sCD163			0.79 (0.72, 0.86)	0.74 (0.67, 0.80)
+ Log-sCD206				0.72 (0.65, 0.78)
Comparison C-index CLIFC-ACLF vs CLIFC-ACLF + 163			0.3267	**0.0328**

carrageenan-induced acute inflammation in rodent models. In the CCl_4-induced liver inflammation and fibrosis model, PXS-4728A was given therapeutically from the halfway point of 4–8 week studies at doses of 2–6 mg/kg in rat or mouse. PXS-4728A improved liver function and reduced the area of fibrosis as measured by picrosirius red staining. In another study, NASH was induced by exposing mice to low-dose streptozotocin and feeding with high-fat diet. PXS-4728A (6 mg/kg oral) was given for the last 3 weeks of a 10 week model. Telmisartan (10 mg/kg) was used as the positive control in this study. PXS-4728A significantly reduced all the components of the NAFLD activity score and collagen deposition, as evidenced by the reduction of picosirius red-positive area.

In a Phase 1 trial PXS-4728A was given orally to healthy male volunteers in a double-blinded, randomised, single ascending dose study. PXS-4728A dose-dependently inhibited plasma SSAO activity for more than 24 hours while the SSAO concentration remained unchanged. In contrast plasma drug concentrations were only measurable for a short period of time consistent with 4728A being an irreversible inhibitor. The safety profile did not identify any signals.

Conclusions: PXS-4728A is an anti-inflammatory and anti-fibrotic drug that reduces the migration of leukocytes and lymphocytes to the site of inflammation and diminishes oxidative stress. PXS-4728A was well tolerated in man and provided significant and long lasting inhibition of enzymatic activity.

LP23

DACLATASVIR PLUS SOFOSBUVIR WITH OR WITHOUT RIBAVIRIN IN PATIENTS WITH HIV–HCV CO-INFECTION: INTERIM ANALYSIS OF A FRENCH MULTICENTER COMPASSIONATE USE PROGRAM

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Introduction: All-oral regimen with daclatasvir (DCV; NS5A replication complex inhibitor) + sofosbuvir (SOF; NS5B polymerase inhibitor) ± weight-based ribavirin (RBV) has demonstrated high sustained virologic response (SVR) rates in HCV mono-infected patients. This analysis reports SVR4 results from an ongoing multicenter compassionate use program (ATU) of DCV+SOF ± RBV in HIV–HCV co-infected patients with advanced liver disease in France.

Material and Methods: Since March 2014, more than 4000 HCV patients from 221 French centers were included in the ATU program. We included HIV–HCV co-infected patients with advanced liver disease who received DCV+SOF QD for 12 or 24 weeks, with RBV added at the physician's discretion. Baseline (start of treatment) characteristics were collected through a standardized form as were follow-up data such as virological response rates and adverse events.

Results: This interim analysis includes a total of 733 co-infected patients, 72.3% males, median age 52.3 years 519 (71.4%) cirrhotic (Child Pugh A=85.3%, B=13.1%, C=1.6%) and 585 (80%) treatment-experienced. Genotype (GT) distribution was as follows: 501 GT1 (69.3%), 2 GT2 (0.3%), 95 GT3 (13.1%), 122 GT4 (17.2%) and 1 GT6 (0.1%). HIV–HBV–HCV triple infection was found in 16 patients. Combined antiretroviral therapy (cART) included nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) in 88.3%, protease inhibitors (PIs) in 35.7%, non-nucleoside reverse-transcriptase inhibitors (NNRTIs) in 24.7% and integrase inhibitors in 61.5% of the patients. 21 patients were not on ART. Baseline median CD4 count was 539/mm^3 (0–1922). RBV was added to DCV+SOF in 98 patients (13.6%). Treatment duration was 24 weeks in 573 (79.3%) and 12 weeks in 142 (19.6%) patients. Overall on 86 patients, SVR4 was obtained in 90% of the cases. Detailed efficacy data are depicted in the table. Treatment discontinuations occurred in 14 patients (1.9%) and were related to an adverse event (n = 4), death (n = 3), patient decision (n = 3), contra-indication (n = 3) and unknown reason (n = 1). Efficacy and safety data will be updated with larger population.

	Treatment duration		Genotype status		
	12 weeks	24 weeks	GT1	GT3	GT4
SVR4	29/35 (83%)	48/51 (94%)	53/60 (88%)	7/8 (88%)	18/19 (95%)

Conclusions: In HIV–HCV co-infected patients with advanced liver disease and treated within the ATU program, DCV+SOF ± RBV regimen was well tolerated and demonstrated high antiviral activity at 4 weeks after the end of treatment.

LP24

SAFETY, EFFICACY AND IMPACT ON LIVER FUNCTION OF SIMEPREVIR (SMV) IN COMBINATION WITH DACLATASVIR (DCV) OR SOFOSBUVIR (SOF) IN PATIENTS WITH SEVERE HEPATITIS C RECURRENCE AFTER LIVER TRANSPLANTATION (LT): RESULTS FROM COMPASSIONATE USE IN EUROPE

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Introduction: SMV has been approved to treat genotype 1 and 4 hepatitis C infection. There are no data on the use of this drug in the treatment of patients with severe hepatitis C recurrence after LT.

Material and Methods: Retrospective analysis of LT recipients with severe hepatitis C recurrence (high risk of death within the next 12 months) receiving SMV as compassionate use in Europe. The aim of this study was to evaluate the safety, efficacy (SVR4) and impact on liver function of SMV-based therapies.

Results: Data from 64 patients is available, 38 patients had cirrhosis, 22 had clinical decompensation and 3 fibrosing cholestatic hepatitis. SMV was administered with SOF or DCV in 41 (64%) or

23 (36%) cases, respectively. Ribavirin (RBV) was used in 35% and 65% of the patients receiving SOF and DCV, respectively. Most of the patients were male (72%) and genotype 1b (81%). Median age was 59 years. Median baseline MELD and Child–Pugh (CPT) scores were 9 and 6, respectively. Among the patients with cirrhosis, 47% were CPT B/C. Tacrolimus was the immunosuppressant used in the majority of the patients (69%). At the beginning of therapy, 20 patients had ascites and 3 had hepatic encephalopathy (HE). Thirty-four patients completed the treatment course and 30 are still on therapy. End of treatment (EOT) response was 88% (30/34) and SVR12 was 83% (25/30). In patients receiving SMV+DCV±RBV, EOT response was 78% (14/18) due to 4 breakthroughs and SVR4 was 72% (13/18) due to an additional relapse by post-treatment week 4. Four of the non-responder patients had cirrhosis and 3 did not receive RBV. In patients receiving SMV+SOF±RBV, EOT (16/16) and SVR12 (12/12) were 100%. At post-treatment week 12 there was a significant improvement in liver function (Table 1). More importantly, ascites and HE resolved in 15 and 2 patients, respectively. SAEs were reported in 11 patients (anemia [n = 2], infections [n = 5], gastrointestinal bleeding [n = 1], renal dysfunction [n = 1], gastric cancer [n = 1] not considered to be related to SMV). One patient presented eosinophilia considered as possibly related to therapy. Rejection or IS-related toxicity were not reported. No major adjustment in IS drugs were needed. None of the patients died or discontinued therapy due to adverse events.

Table 1. Evolution of liver tests during and after antiviral therapy

Variable (n = 26)[a]	Baseline	On-treatment W12	PTW12	*p*(Baseline vs. PTW12)
Bilirubin (mg/dL)	1 (0.3–4.2)	1.3 (0.4–4.6)	0.8 (0.1–4.8)	0.007
INR	1.1 (0.95–1.85)	1.1 (0.84–2.1)	1.1 (0.93–1.57)	0.308
AST (IU/L)	89 (15–548)	25 (8–83)	28 (14–77)	0.001
ALT (IU/L)	77 (7–296)	20 (7–140)	22 (9–109)	0.001
MELD score	9 (6–18)	10 (6–20)	8 (6–18)	0.006
Child–Pugh score	6 (5–10)	5 (5–11)	5 (5–7)	0.023

Data are expressed as median (range). PTW12, post-treatment week 12.
[a] Patients with available data at PTW12.

Conclusions: SMV in combination with DCV or SOF is effective and safe in patients with severe HCV recurrence after LT. Viral eradication is associated with a fast and significant improvement in liver function.

LP25
ADD-ON PEGINTERFERON ALFA-2A SIGNIFICANTLY REDUCES HBsAg LEVELS IN HBeAg-NEGATIVE, GENOTYPE D CHRONIC HEPATITIS B PATIENTS FULLY SUPPRESSED ON NUCLEOT(S)IDE ANALOGUE TREATMENT: THE HERMES STUDY

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Introduction: Long-term treatment with nucleos(t)ide analogues (NA) efficiently suppresses viral replication in patients with HBeAg-negative chronic Hepatitis B (CHB), yet minimally affecting serum HBsAg levels. We investigated whether add-on PEG-IFN fosters qHBsAg kinetics in NA suppressed, HBeAg-negative, genotype D, Caucasian CHB patients (HERMES study).

Material and Methods: This phase IIb, open-label, single-arm, multicenter study conducted in 13 sites in Italy included patients who following NA monotherapy, had serum HBV-DNA persistently <20 IU/ml for at least 12 months, HBsAg >100 IU/ml and stable qHBsAg in the 3 months preceding enrollment (<0.5 $\log_{10}$ IU/ml decline). Patients without any HBsAg decline at week 24 or those who completed the add-on treatment period (week 48), discontinued PEG-IFN and were followed-up for additional 48-weeks. Serum HBsAg decline at the end of treatment with PEG-IFN plus NA combination (week 48) was the primary endpoint.

Results: 70 Caucasians started treatment with PEG-IFN. Median age was 50.5 years (range 29–64), 81% were males, 93% were on tenofovir or entecavir, viral suppression lasting for 3.2 years (range 1.1–8.4). Baseline stiffness by FibroScan was 6.1 kPa (range 3.3–12.7) in the 30 tested patients. Median HBsAg levels significantly decreased from 1160 IU/ml at baseline to 308 IU/ml at week 48 ($p < 0.0001$, Table). At week 48 (primary endpoint), serum HBsAg decreased ≥50% from baseline in 30 patients (43%). Likewise, the proportion of patients with HBsAg <1000 or <500 or <100 IU/ml increased from baseline up to week 48 (Table). One patient (1.4%) seroconverted to anti-HBs at week 24 and successfully withdrew from both NUC and IFN treatment and two additional patients (2.8%) reached qHBsAg <10 IU/ml. Sixty-two patients (88.6%) experienced adverse events (AEs), 8 (11.4%) and 7 (10.0%) patients discontinued or temporarily interrupted PEG-IFN due to AEs respectively, while in 8 (11.4%) patients PEG-IFN was downdosed following AEs. Two (2.9%) patients had serious AEs related to PEG-IFN (pyrexia and haemoptysis). Overall, 24 patients (34.3%) discontinued PEG-IFN, 11 (16%) HBsAg non responders, 8 (11%) for AEs, 5 (7.1%) for compliance or consent withdrawal.

Conclusions: In HBeAg-negative, genotype D, CHB patients treated with NA, add-on treatment with PEG-IFN results in a significant reduction of serum HBsAg levels.

LP26
NUCLEIC ACID POLYMERS ARE EFFICIENT IN BLOCKING HEPATITIS DELTA VIRUS ENTRY IN VITRO

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Introduction: Nucleic acid polymers (NAPs) are phosphorothioated oligonucleotides, which exhibit a sequence-independent, broad-spectrum antiviral activity. NAPs have been previously shown to have antiviral effect against duck hepatitis B virus (DHBV) infection in vitro, having both entry and post-entry activities (Noordeen et al., 2013). Additionally NAPs have been shown to be clinically active against HBV infection where they achieve rapid reductions in both serum HBsAg and HBV DNA.

Material and Methods: HBV and hepatitis delta virus (HDV) are considered to have similar entry mechanisms, therefore the antiviral activity of various NAP compounds was assessed against HDV infection using two *in vitro* cellular infection models.

Table (abstract LP25): HBsAg kinetics during 48 weeks of PEG-IFN and NA combination treatment

	Baseline (n = 70)	Week 12 (n = 67)	Week 24 (n = 66)	Week 36 (n = 50)	Week 48 (n = 46)
HBsAg (IU/mL), median (range)	1160 (133.3–8909)	1317 (1.6–8158)	743 (0.04–5507)*	406 (3.2–6027)	308 (0.5–5202)*
HBsAg <1000 IU/mL, N (%)	33 (47%)	32 (48%)	39 (59%)	34 (68%)	33 (72%)
HBsAg <500 IU/mL, N (%)	16 (23%)	20 (30%)	26 (39%)	24 (48%)	26 (56%)
HBsAg <100 IU/mL, N (%)	0 (0.0%)	3 (4.5%)	9 (14%)	8 (16%)	10 (22%)

*$p < 0.0001$ vs baseline (Wilcoxon Signed Rank test).

The NAPs tested included REP 2006 (a prototypic NAP with a degenerate sequence), REP 2055, REP 2139 (clinically active against HBV) and REP 2165 (an additional clinical candidate NAP). Differentiated HepaRG cells, or NTCP-expressing Huh-7 cells, were inoculated with HDV in the presence of NAPs, and infection was monitored by measuring intracellular HDV RNA levels at day-9 postinoculation. Control experiments consisted of NAP treatment applied postinoculation to specifically monitor for toxicity and inhibition of HDV RNA replication.

Results: Treatment with NAPs resulted in the absence of cytotoxicity at ≤10 μM concentrations. All NAPs demonstrated a dose dependent activity against HDV infection in both HepaRG and NTCP-Huh-7 cells, with an IC50 ≤625 nM for the prototypic NAP. Data show that the antiviral effect was exerted at viral entry and not on HDV RNA replication. The antiviral effect of the prototypic, degenerate REP 2006 was comparable to that of other NAPs with defined sequences.

Conclusions: NAPs display an antiviral activity against HDV entry in vitro, which has the same sequence-independent antiviral effect as that observed for DHBV in vitro. These results suggest that NAPs are potent inhibitors of HDV infection.

LP27

ACH-3422, A NOVEL NUCLEOTIDE PRODRUG INHIBITOR OF HCV NS5B POLYMERASE

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Introduction: ACH-3422 is a nucleotide inhibitor of hepatitis C virus (HCV) NS5B RNA polymerase, displaying pan-genotypic activity and a high *in vitro* barrier to resistance. This randomized, double-blind, placebo (PBO)-controlled study evaluated the safety, tolerability, pharmacokinetics (PK), and antiviral activity of ACH-3422 after single ascending doses (SAD) and multiple-ascending doses (MAD) in healthy volunteers (HV) and antiviral proof of concept (POC) in patients (pts) infected with genotype 1 (GT-1) HCV.

Material and Methods: This was a 3-phase study whereby 5 different doses of ACH-3422 (50 mg, 150 mg, 300 mg, 500 mg and 700 mg) and PBO were administered in a SAD and 14-day MAD phase with HV, and either a 7 or 14-day POC phase in pts with HCV GT-1. Dose escalation decisions were made in real time based on the emerging safety, PK, and virology assessments.

Results: 104 HV (18–49 years; 73 male) and 37 HCV (IL28 frequency: CC 9, CT 24, TT 4; 27–69 years; 26 male) were randomized. In the SAD/MAD phases, ACH-3422 was well tolerated: no serious adverse events (SAEs), no discontinuations due to adverse events (AEs), no grade 3–4 AEs, and no clinically significant AEs or laboratory or ECG abnormalities were observed. In the POC phase, ACH-3422 was well tolerated: no SAEs or Grade 3 or 4 AEs were considered drug related. No Grade 3 or 4 laboratory abnormalities were observed among active pts, and one discontinuation due to an AE was observed in a PBO patient. There was no significant difference between active and PBO groups in AEs or laboratory abnormalities, and no clinically significant ECG abnormalities were observed. Among active pts, increasing doses of ACH-3422 resulted in increasing viral decline (Figure). In the 700 mg POC group, a mean maximum viral load drop of 3.4 $\log_{10}$, 4.2 $\log_{10}$, and 4.6 $\log_{10}$ was reported after 7, 10, and 14 days of treatment, respectively. Three of 6 pts (50%) achieved viral clearance (target not detected by PCR) with 14 days of 700 mg. Approximate dose-proportional PK was observed for both ACH-3422 (50–700 mg QD) and its corresponding nucleoside metabolite (ACH-3420).

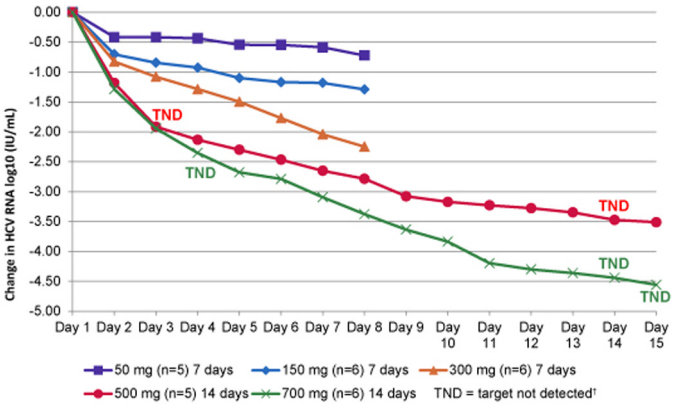

Figure: Mean maximum change in viral load. Maximum change = maximum reduction from Baseline (Day 1 Hour 0) in HCV RNA as of that time point. †2 subjects achieved TND in the 500 mg group; 3 subjects achieved TND in the 700 mg dose group.

Conclusions: In all 3 phases, ACH-3422 was safe and well tolerated at doses up to 700 mg with dose-related virologic responses in pts with HCV GT-1 infection. Together, these results support further investigation of ACH-3422 with ACH-3102, a potent NS5A inhibitor, for the treatment of pts with HCV GT-1 infection.

LP28

EFFICACY OF THE ORAL SOFOSBUVIR-BASED COMBINATIONS IN HCV GENOTYPE 4-MONO-INFECTED PATIENTS FROM THE FRENCH OBSERVATIONAL COHORT ANRS CO22 HEPATHER*

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Introduction: Real-life results of the Sofosbuvir (SOF)/Simeprevir (SIM) combination have been extensively reported in genotype 1-infected patients but there are few or no data regarding genotype 4-infected patients for the SOF/SIM or the SOF-Daclatasvir (DCV) combinations. In January 2015, more than 3003 patients of the French observational cohort ANRS CO22 HEPATHER were given the new oral antivirals in 32 centers: we report the preliminary results of the Sofosbuvir-based combinations with DCV or SIM, with or without ribavirin (RBV), in Genotype 4-infected patients.

Material and Methods: Demographics, history of liver disease were collected at entry in the cohort. Clinical, adverse events, and virological data were collected throughout treatment and post-treatment follow-up.

Results: 81 (64 cirrhotic) genotype 4-infected patients were given a combination of 1. SOF (400 mg/d) + DCV (60 mg/d) in 47 patients including 15 patients with ribavirin (1–1.2 g/d) for 12 (n = 11) or 24 weeks (n = 36); 2. SOF (400 mg/d) + SIM (150 mg/d) in 34 patients including 7 patients with ribavirin (1–1.2 g/d) for 12 (n = 23) or 24 weeks (n = 11). Results are given in the table.

The overall SVR4 rate was high from 78 to 100% according to the baseline characteristics and the therapeutic schedule. There was no clear difference between the SOF/DCV or the SOF/SIM combination and there was an additive effect in cirrhotic or experienced patients of either extension of the duration of therapy from 12 to 24 weeks or the addition of RBV, for the 2 combinations. The 12 week combination of SOF/DCVRBV or SOF/SIM/RBV achieved a 100% SVR4 rate in cirrhotics without additive effect of extension of the treatment to 24 weeks and this was also observed in experienced patients; all non-cirrhotic patients achieved 100%SVR4. The 12 week combination of SOF/DCV or SOF/SIM is a good therapeutic option and is generally well tolerated.

	SOF/DCV (n = 32)		SOF/DCV/RBV (n = 15)		SOF/SIM (n = 27)		SOF/SIM/RBV (n = 7)	
Tx duration (weeks)	12 w.	24 w.	12 w.	24 w.	12 w.	24 w.	12 w.	24 w.
% SVR4	88.9	94.7	100.0	100.0	78.3	100.0	100.0	100.0
% SVR12	100.0	90.9	100.0	100.0	88.9	100.0	100.0	100.0
% SVR4 cirrhotic	85.7	92.3	100.0	100.0	69.2	100.0	100.0	100.0
% SVR4 non-cirrhotic	100.0	100.0	–	100.0	100.0	–	–	–
% SVR4 naïve	100.0	100.0	–	100.0	66.7	100.0	100.0	–
% SVR4 experienced	87.5	94.1	100.0	100.0	77.8	100.0	100.0	100.0

Conclusions: The 12 week combination of Sofosbuvir–Daclatasvir or Sofosbuvir–Simeprevir is associated with a high rate of SVR4 in genotype 4-infected patients. The addition of Ribavirin increases the SVR rate in cirrhotic or experienced patients at 12 weeks without additive effect of the treatment extension to 24 weeks.

*The ANRS CO22 Hepather cohort is supported by MSD, Janssen, Gilead, BMS, Roche, Abbvie and conducted in collaboration with AFEF.

LP29

A RANDOMISED PROSPECTIVE OPEN-LABEL TRIAL COMPARING PEGINTERFERON + ADEFOVIR AND PEGINTERFERON + TENOFOVIR VERSUS NO TREATMENT IN HBeAg NEGATIVE CHRONIC HEPATITIS B PATIENTS WITH LOW VIRAL LOAD: ANALYSIS OF WEEK 48 RESULTS

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Introduction: Chronic hepatitis B (CHB) patients with a low viral load (LVL) are currently not eligible for antiviral treatment. However, they comprise the largest group of hepatitis B virus-infected patients and are still at risk to develop cirrhosis or hepatocellular carcinoma. Here we present the week 48 results of a randomized trial comparing combination treatment of peginterferon alfa-2a (Peg-IFN) and a nucleotide analogue versus no treatment for CHB patients with LVL.

Material and Methods: 134 CHB patients (HBeAg-negative, HBV-DNA <20,000 IU/mL) were randomized 1:1:1 to receive Peg-IFN+adefovir (arm I; n = 46), Peg-IFN+tenofovir (arm II; n = 45) or no treatment (arm III; n = 43) for 48 weeks (ITT population).

Randomization was stratified by HBV genotype A (22%), non-A (B 7%, C 4%, D 26%, E/F/G 20%), or indeterminate (21%). The median age was 43 years, 57% were male. Twelve patients discontinued the study before week 48 (5 in arm I, 6 in arm II, 1 in arm III). HBsAg loss (AxSYM <0.05 IU/mL) and quantitative HBsAg level (Architect) was determined at regular intervals, and were compared using Fisher's, Mann–Whitney U or Wilcoxon test.

Results: At week 48, 4 patients receiving combination therapy had achieved HBsAg loss, compared to none of the untreated patients (ITT 4.4% vs 0.0%, p = 0.31). Patients with HBsAg loss were treated in arm I (n = 1) and arm II (n = 3), and had HBV genotype A (n = 1), B (n = 1), or indeterminate (n = 2). Baseline HBsAg levels were comparable between study arms (median 3.34 log IU/mL). In a per-protocol analysis, HBsAg level had declined significantly in all arms at week 48; −0.33 (p < 0.001), −0.22 (p < 0.001), and −0.07 (p = 0.02) median log reduction for arms I, II, and III, respectively. No difference in HBsAg decline was observed between treatment arms. However, HBsAg declined more in treatment arms I (p < 0.001) and II (p = 0.002) compared to the control arm III. A strong HBsAg decline of >1.0 log IU/mL was observed in 17 treated patients (21%), but in none of the untreated patients (p < 0.001). No unexpected adverse events were observed in the treatment arms.

Conclusions: In CHB patients with a low viral load, 48 weeks of combination treatment with Peg-IFN and adefovir or tenofovir resulted in 4.4% HBsAg loss, compared to 0.0% in the untreated control group. The significant decline in HBsAg at week 48 may indicate a further increase in the rate of HBsAg loss during treatment-free follow-up. Week 72 results are expected in April 2015.

LP30

LIVER-DIRECTED ALLOSTERIC INHIBITORS OF ACETYL-CoA CARBOXYLASE FAVORABLY IMPACT PATHOPHYSIOLOGY IN THE PROGRESSION FROM NAFLD TO NASH AND HEPATOCELLULAR CARCINOMA, INCLUDING HEPATIC STEATOSIS, INFLAMMATION, AND FIBROSIS

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Introduction: Dysregulated fatty acid (FA) metabolism occurs via elevated FA synthesis (FASyn), impaired FA oxidation, or both. Acetyl-CoA Carboxylase (ACC) occupies a unique position in FA metabolism, responsible for both increased FASyn and reduced FA oxidation. Pharmacologic inhibition of ACC presents an attractive mode for reducing de novo lipogenesis and enhancing FA oxidation. It has been proposed that hepatic steatosis leads to inflammation and production of toxic lipid oxidation byproducts that drive development of fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). We sought to determine the impact of liver-directed, selective ACC inhibition on the pathological processes involved in the progression from hepatic steatosis to NASH and HCC.

Material and Methods: Recently identified selective allosteric inhibitors of ACC, ND-630 and ND-654, were evaluated to determine the effects of ACC inhibition on the specific pathologies associated with NASH and HCC including hepatic de novo lipogenesis, inflammation, and fibrosis. ND-630 and ND-654 inhibit HepG2 FASyn with EC_{50} of 8.7nM and 4 nM, and hepatic FASyn in vivo with ED_{50} of 0.14 mg /kg and 0.3 mg /kg, respectively.

Results: Chronic inhibition of ACC with ND-630 in an in vivo model of high sucrose diet-induced obesity in rats led to potent inhibition of plasma triglycerides, cholesterol, and free fatty acids, and reduced hepatic triglyceride levels with maximal inhibition on Day 14 at 10 mg /kg. Emerging data demonstrate that ND-654 has potent activity to inhibit tumor formation and prolong survival in the diethylnitrosamine (DEN) model of HCC in male Wistar rats treated weekly with 50 mg /kg DEN to induce sequential development of fibrosis, cirrhosis, and HCC. Importantly, ND-654 significantly (p < 0.01) inhibited HCC development in DEN-injured livers from an average of 15.8 tumor nodules in controls to an average of 7.1 tumor nodules in ND-654-treated rats and prolonged survival by several weeks. Interestingly, recent mechanistic studies in the DEN model of HCC demonstrated that inhibition of ACC produced a dose-dependent decrease in fibrosis as assessed by the collagen proportional area (CPA) of Sirius Red stained histological sections, and a decrease in mRNA for markers of inflammation including α-SMA (a marker of stellate cell activation) and CD-68 (a marker of macrophage infiltration).

Conclusions: These data demonstrate that liver-directed inhibition of ACC can impact multiple steps in the pathophysiological cascade during the progression from fatty liver disease to the more advanced diseases of NASH and hepatocellular carcinoma.

LP31

STRENGTHENING VIRAL HEPATITIS SURVEILLANCE IN EUROPE: RECOMMENDATIONS FROM TWO GLOBAL HEPATITIS POLICY SURVEYS (2013 AND 2014)

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Introduction: Weak surveillance is a key barrier to evidence-based and country context-adapted responses to growing hepatitis B and C epidemics. We describe surveillance capacity and identify areas where improvements are urgently needed as reported by governments and civil society (CS) from Member States (MS) of the WHO European Region.

Material and Methods: A descriptive and bivariate (comparison between EU/EEA and non-EU/EEA countries) analysis of responses to surveillance-related questions from the WHO Global Hepatitis Policy Survey (2013) was performed and complemented by analysis of corresponding CS responses collected through the Global Community Hepatitis Policy Survey (2014).

Results: 44 of the 53 European MS (83%) responded to the Global Hepatitis Policy Survey and responses from CS were available for 35 countries. Less than one-third (27%) of MS reported having written national strategies containing a surveillance component. While 43 MS (98%) reported the existence of routine viral hepatitis surveillance, only 64% had a national surveillance system for chronic hepatitis B and 61% for chronic hepatitis C. Moreover, in 11 countries (34%) CS respondents disagreed with their governments on this matter. 80% of MS reported having national registries for liver cancer cases and 66% for HIV/hepatitis co-infection. Although 89% of responding MS reported publishing hepatitis reports regularly, this was challenged by 51% of CS responses. Only 45% of MS reported conducting regular viral hepatitis sero-surveys. Of 32 responding countries implementing universal newborn first dose hepatitis B immunisation, 28% reported that data on vaccination coverage for newborns were not available. Areas in which governments would like assistance from WHO included surveillance (23%), viral hepatitis national burden estimation (34%) and development of national plans for viral hepatitis prevention and control (39%), with a higher proportion of non-EU/EEA countries desiring assistance (p = 0.01, p = 0.097 and p = 0.024, respectively).

Conclusions: Many countries in the WHO European Region face limitations in conducting chronic viral hepatitis disease surveillance, assessing the burden of disease and measuring the impact of interventions. Recent treatment advances make it all the more imperative to mobilise political will to develop comprehensive

national strategies supported by the upcoming WHO Global Strategy on Hepatitis (2016–2021) and technical assistance to MS.

LP32

REAL-WORLD EFFECTIVENESS OF LEDIPASVIR/SOFOSBUVIR 8 WEEKS CHRONIC HEPATITIS C TREATMENT

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Introduction: Ledipasvir/Sofosbuvir (LDV/SOF) single tablet regimen (STR) is approved in Europe for the treatment of chronic hepatitis C (CHC) patients with genotypes (GT) 1, 3 and 4. The ION-3 study showed that 8 weeks (8w) of LDV/SOF treatment was non-inferior to 12 weeks in previously untreated GT1 patients without cirrhosis with no benefit for the addition of Ribavirin. According to the SPC 8w may be considered in this population. The aim of the present analysis is to characterise the population receiving 8w LDV/SOF and to describe outcomes in clinical practice.
Material and Methods: The first CHC patients treated with 8w LDV/SOF in a single centre in Germany, and for whom sustained virological response after 4 weeks of follow-up (SVR4) will be available in April, were included in the analysis. Baseline characteristics, prior treatment history, safety and effectiveness were investigated. The analysis was performed using descriptive statistics.
Results: 46 patients met the inclusion criteria for this analysis. These patients initiated 8w treatment with LDV/SOF between 24/11/2014 to 27/01/2015. No patient had ribavirin added to the STR. The mean (SD) age was 50.9 (12.4) years and 56.5% were males. The genotype distribution was 52%, 44% and 4% for GT1a, GT1b and GT4, respectively. At entry, 98% of patients had no cirrhosis, one patient had compensated disease. The METAVIR stage distribution of non-cirrhotic patients at baseline was 39.1%, 32.6%, 19.6% and 8.7% for F0, F1, F2 and F3, respectively. Median (range) HCV RNA at baseline was 5.86 (Q1-Q3 5.38–6.22; Min-Max 3.74–6.67) $\log_{10}$ IU/ml, no patient had HCV RNA ≥6 million IU/mL. No patient was HIV co-infected and one patient was HBV co-infected. Overall, 98% of the patients were treatment-naïve. One patient had relapsed after previous IFN/RBV therapy. At baseline, co-morbidities were reported in 93% of patients, with depression (16%) and arterial hypertension (16%) being most common. Up to date, no discontinuations or relevant Adverse Drug Reactions have been observed. Complete results regarding SVR4, adverse events and discontinuations will be available at the time of presentation.
Conclusions: 8w LDV/SOF is predominantly prescribed according to the SPC for treatment-naïve non-cirrhotic CHC patients with HCV RNA <6 million IU/mL at baseline. Preliminary results indicate that LDV/SOF is a safe, well tolerated treatment option with no adverse drug reactions or discontinuations reported so far.

LP33

EFFICACY OF INTERFERON +/− NUCLEOS(T)IDE ANALOG TREATMENT IN CHILDREN WITH CHRONIC HEPATITIS B, IN THE IMMUNOTOLERANT PHASE CONFIRMED BY LIVER BIOPSY

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Introduction: To investigate the efficacyof interferon (IFN) monotherapy or in combination with nucleos(t)ide analogs (NA) in pediatric patients with chronic hepatitis B (CHB), in the immunotolerant phase.
Material and Methods: Ninety-sevenchildren with CHB (HBeAg-positive and HBV-DNA-positive) aged <18 yrs in the immunotolerant phase were included in the analysis. Immunotolerance was defined as low necroinflammatory activity (G ≤ 1 on liver biopsy, Scheuer score) and ALT <2×ULN (40 IU/L). Patients with HBV-DNA >7 log IU/mL received IFN (IFNα 3–5 MIU/m^2 × body surface area once daily, PegIFNα-2a 104 μg/m^2/wk or PegIFNα-2b 1.5 μg/m^2/wk) plus lamivudine (3 mg/kg/day) or adefovir (2–6 yrs: 0.3 mg/kg/day; 7–11 yrs: 0.25 mg/kg/day; >12 yrs: 10 mg/day). Patients with HBV-DNA ≤7 log IU/mL received IFN monotherapy for 12 wks; if, at Wk 12, HBV-DNA declined ≥2 log IU/mL, treatment was continued, but if HBV-DNA declined <2 log IU/mL, patients received IFN plus lamivudine or adefovir. Treatment duration ranged between 72–144 weeks, with a follow-up period of 24 wks post-end of treatment (EOT).
Results: Majority of patients were male (69%) with a mean age of 7.8 years and mean HBV-DNA of 8.76 IU/mL. Eighteen patients (18.6%) received IFN-monotherapy and 79 (81.4%) received IFN/NA combination therapy. At baseline, 39 (40%) patients had ALT levels <ULN and 58 (60%) between 1–2×ULN withHBeAg seroconversion rates of 56.4% (22/39) and 63.8% (37/58) respectively and HBsAg negative/seroconversion were 33.3% (13/39) and 39.7% (23/58) respectively. A summary of virologic response for all subjects during treatment and follow-up are shown in Table 1. Mean treatment duration was shorter among those who relapsed to HBsAg-positivity during follow-up vs. non-relapsers (99±21 vs. 131±20 wks; p = 0.005). Younger age (1–6 vs. 7–17 yrs) was significantly associated with achieving HBeAgor HBsAgseroconversion (p < 0.05). ALT levels (<1×ULN vs. 1–2×ULN) and history of preventing the vertical transmissionwere not significantly associated with efficacy. The main adverse events included pyrexia (68%), neutropenia (51%, mild), rash (13%), hypothyroxinemia (13%), hyperthyroxinemia (11%), and fatigue (10%).
Conclusions: IFN (IFNα or PegIFNα) monotherapy or in combination with NAs is an effective treatmentin pediatric patients in the immunotolerant phase of CHB.

LP34

EX VIVO LIVER RESECTION AND AUTOTRANSPLANTATION: A FEASIBLE AND HIGHLY EFFECTIVE PROCEDURE FOR PATIENTS WITH END-STAGE ALVEOLAR ECHINOCOCCOSIS – 15 CASES EXPERIENCE

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Introduction: The role of *ex vivo* liver resection and autotransplantation in end-stage hepatic alveolar echinococcosis (HAE) is unclear. We aimed to present our experience and propose a selection criteria for this unique technique in patients with end-stage HAE.
Material and Methods: Fifteen patients with end-stage HAE were considered unresectable by conventional resection due to critical invasion to hepatocaval region along with three hepatic veins and retrohepatic vena cava, as well as the tertiary portal and arterial branches. Detailed information was tabulated and the possible criteria was proposed.
Results: All the 15 patients successfully underwent *ex vivo* extended right hepatectomy and autotransplantation without intra-operative mortality. Synchronous lung lobectomy was performed in two cases. The median autograft weight was 706 g (380–1000g); operative time 15.5 hours (11.5–20.5 hours); anhepatic time 283.8

minutes (180–435 min). Median blood transfusion requirement was 7.9 units (0–15 units). Postoperative hospital stay was 32.3 days (12–60 days). Post-operative complication Clavien-Dindo grade IIIa or higher occurred in three patient including one death occurred 12 days after the surgery due to acute liver failure. One patient had lost follow-up after six month. Thirteen patients were followed-up with median period of 21.6 months with no relapse.

Conclusions: This is the largest reported series of patients with end-stage HAE treated with liver autotransplantation. The technique requires neither organ donor, nor post-operative immune suppressant. The early postoperative mortality was low with acceptable morbidity. Preoperative precise assessment and strict patient selection are utmost important.

LP35

A PLACEBO-CONTROLLED, MULTICENTER, DOUBLE-BLIND, RANDOMISED, PHARMACOKINETIC AND PHARMACODYNAMIC TRIAL OF EMRICASAN (IDN-6556) IN SUBJECTS WITH ACUTE-ON CHRONIC LIVER FAILURE (ACLF)

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Introduction: ACLF is characterized by acute deterioration of liver function in subjects with liver cirrhosis. Commonly precipitated by an acute event, it is associated with extra-hepatic organ failure leading to high in-hospital mortality dependent on severity despite maximal support of failing organs. Serum markers of caspase-driven apoptosis are raised in ACLF subjects. Emricasan is an orally active caspase inhibitor with demonstrated efficacy in animal models of liver disease. Emricasan inhibits caspases involved in the maturation of pro-inflammatory cytokines, IL-1β and IL-18. We studied the PK/ PD and safety of emricasan and its effect on caspase activity and apoptosis in subjects with ACLF.

Material and Methods: 21 Subjects with ACLF-1 and -2 were randomised to receive oral emricasan 5 (n = 5), 25 (n = 7) and 50 mg (n = 5), or pbo (n = 4) BID for 28 days. Full PK/PD profiles were obtained on Days 1, 4 and 28. Clinical responders as defined in the table were evaluated at Day 28 or last known follow up.

Results: 7 subjects completed the study: pbo (n = 2), 25 (n = 3) and 50 mg (n = 2). 6 subjects died [pbo (n = 2), 5 (n = 1), 25 (n = 1) and 50 mg (n = 2) respectively] by Day 28; 13/21 subjects reported serious AEs, none of which were considered treatment related. Emricasan blood levels were increased at all doses compared to previous patient populations studied. Baseline values of cCK18 and Caspase 3/7 were significantly higher in ACLF compared to previous populations studied. Clinical responders generally experienced at least 30% reduction in cCK18 and rises in CK18 during the study were generally associated with disease related adverse events. Oral emricasan 25 and 50 mg BID reduced cCK18, flCK18 and Caspase 3/7 levels within 24h post dose by at least 30% in most subjects but only the 50 mg group experienced sustained reductions in cCK18 over the entire dosing period in the majority of subjects. The median reduction in the 50 mg group on Day 1 was 54% compared with 7, 13 and 44 in the pbo, 5 and 25 mg groups respectively.

Conclusions: Biomarkers of apoptosis and caspase activity (cCK18, flCK18, Caspase 3/7) were implicated in ACLF and disease severity, and appeared associated with patient outcome. Emricasan was well tolerated. Increases in cCK18 levels appeared to be associated with disease-related adverse clinical events. The combined PK/PD data suggest higher doses of emricasan may be required in ACLF in future studies to achieve sustained reductions in disease relevant biomarkers.

LP36

UNDERSTANDING HEPATITIS DELTA VIRUS AND HBsAg KINETICS DURING TREATMENT WITH PRENYLATION INHIBITOR LONAFARNIB VIA MATHEMATICAL MODELING

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Introduction: The new prenylation inhibitor lonafarnib (LNF) is a potent antiviral agent that provides a breakthrough for the treatment of HDV and an opportunity to further characterize HDV and hepatitis B surface antigen (HBsAg) dynamics during effective treatment with an oral anti-HDV agent.

Material and Methods: HDV RNA and HBsAg kinetic data were obtained from a Phase 2a study in which 12 patients were treated with LNF 100 mg twice daily (n = 6, Group 1) or 200 mg twice daily (n = 6, Group 2) for 28 days. Blood samples were

Table (abstract LP35): Clinical responders

Criteria	Treatment group assignment				
	Placebo (n = 4)	5 mg BID (n = 5)	25 mg BID (n = 7)	50 mg BID (n = 5)	All subjects (n = 21)
Survival at Day 28 or last known follow-up	2	3	3	3	11
CLIF-SOFA score (improvement by 2 points from baseline)	1	3	2	1	7
MELD score (improvement by 5 points from baseline)	1	2	3	2	8
Total bilirubin (improvement by ~50% from baseline)	2	3	2	1	8
Number of responders	2	3	3	3	11

collected frequently during the first 72 hr, and then weekly until day 28. Viral decline slopes and transition time were estimated by segmented linear regression. A dual mathematical model (Hepatology 2014;60:1902–10) that couples both HDV and HBsAg kinetics was used for the estimation of the LNF pharmacological delay, t_0, HDV clearance rate (c) and LNF effectiveness in blocking HDV production, ε.

Results: The median pretreatment serum HDV RNA and HBsAg levels were estimated as 5.97 (standard error, se = 0.13) log IU/mL and 4.17 (se = 0.12) log ng/mL. While HBsAg remained unchanged, a biphasic decline in HDV RNA during therapy was characterized in all LNF-treated patients, with a first rapid decline phase followed by a second slower decline (or plateau) phase. A short pharmacological delay of t_0=0.73 (se=0.24) day, in which HDV remained at baseline levels, was not associated with LNF dose. The HDV clearance rate in the serum, *c*, was estimated as 0.426 (se=0.04) d^{-1}, corresponding to an HDV half-life of 1.63 (se=0.15) days. LNF effectiveness in blocking HDV production was significantly ($p < 0.001$) higher in Group 2 [ε = 0.952 (se = 0.057)] than Group 1 [ε = 0.739 (se = 0.05)].

Conclusions: The observed stable HBsAg level during LNF treatment suggests that the productively HDV-infected cell number remained unchanged during this relatively short treatment period. The effectiveness of the 200 mg LNF dose in blocking HDV production (ε = 95%) is similar to that previously estimated in patients treated with pegIFN (ε = 96%) [Hepatology 2014; 60: 1902–10]. A strikingly shorter delay was observed with LNF (t_0 = 0.73 day) compared to HDV-infected patients treated with pegIFN (t_0 = 8.5 day), and the refined estimate of HDV $t_{1/2}$ = 1.4 day was about 2-fold shorter than under pegIFN ($t_{1/2}$ = 2.9 day). These findings suggest that LNF might have two mechanisms of action – blocking viral production and assembly/secretion, reminiscent of the dual mechanism of action observed with an HCV NS5A inhibitor [PNAS 2013; 110: 3991–6].

LP37

A PLACEBO-CONTROLLED, MULTICENTER, DOUBLE-BLIND, RANDOMISED TRIAL OF EMRICASAN IN SUBJECTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND RAISED TRANSAMINASES

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Introduction: NAFLD and its inflammatory/fibrotic subgroup of non-alcoholic steatohepatitis (NASH), can be considered the hepatic manifestations of the metabolic syndrome and is perhaps the most common cause of chronic liver disease in the US. NASH can progress to cirrhosis, liver failure and increased risk of hepatocellular cancer. Currently, no drugs are approved for the treatment of liver disease associated with NAFLD or NASH. Emricasan (IDN-6556) is an orally available irreversible pan-caspase inhibitor. Caspases are proteases responsible for executing apoptotic pathways, or programmed cell death, and for activation of cytokines (IL-1β and IL-18). Both caspase-mediated apoptosis and inflammation have been shown to play important roles in the development and progression of liver disease associated with NAFLD and NASH. Emricasan has demonstrated efficacy in a number of animal models of liver disease and was also protective in models of NAFLD/NASH where it reduced steatosis, inflammation, apoptosis and fibrosis, suggesting disease-modifying potential. The current study evaluated safety and the impact of emricasan treatment on generic and mechanism specific biomarkers in NAFLD/NASH subjects with elevated liver enzymes.

Material and Methods: Thirty eight subjects with proven NAFLD/NASH and raised ALT (≥1.5×ULN on 2 occasions at least 7 days apart) were entered into the study and randomly assigned to receive oral emricasan 25 mg BID or placebo for 28 days. Subjects were then followed up for an additional 28 days post study for safety assessment. Subjects on statins, fibrates, sulfonylureas and/or metformin were required to be on stable doses of drug for 3 months prior to study entry and for the study duration. The primary endpoint in the study was change from Baseline in ALT at Day 28. Key secondary endpoints included change from Baseline in AST, cCK18, flCK18, caspase 3/7 and HOMA-IR.

Results: All subjects completed the study and the database will be locked in March 2015. The study results will be available for presentation at the meeting.

Conclusions: Effects of emricasan on raised transaminases, caspase activity and markers of apoptosis in subjects with NAFLD/NASH will be discussed in the context of the potential to use this etiology-specific response information to guide future studies in subjects with more advanced liver disease.

LP38

EFFICACY AND SAFETY OF IFN-FREE ANTIVIRAL THERAPY IN PATIENTS WITH HCV RECURRENCE AFTER LIVER TRANSPLANTATION: REAL-LIFE EXPERIENCE FROM AUSTRIA

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Introduction: Patients with hepatitis C virus (HCV) recurrence after orthotopic liver transplantation (OLT) have a high need of antiviral therapy due to accelerated progression of the disease. IFN-free regimens revealed promising efficacy and safety data in clinical studies. We evaluated real-life experience of efficacy and safety of all-oral regimens in patients with HCV-recurrence after OLT.

Material and Methods: 77 OLT-patients and 5 with liver/kidney-transplantation started an all-oral anti-HCV treatment. 68 beyond treatment-week 4 were analyzed (male: 54/79%, age: 59±9 [33–78] years, GT-1: 51 [1a: 13; 1b: 35; 1g: 1, no subtype: 2]; GT-3a: 10; GT-4: 6 [h: 1]; missing: 1). Time from OLT to treatment was 77±67 months. 7 patients had fibrosing cholestatic hepatitis (FCH), 37 cirrhosis (CPS-A: 31; CPS-B/C: 6), 47/69% were treatment-experienced. 53/78% patients received immunosuppression with calcineurin-, the others with mTor-inhibitors.

Treatment regimens were: Sofosbuvir (SOF) with ribavirin (RBV): 18 (27%), SOF/Daclatasvir (DCV): 39, SOF/Simeprevir (SIM): 7, SOF/Ledipasvir (LDV): 3; one patient received SIM/DCV. HCV-RNA-quantification was performed with Abbott Real*Time* HCV ([ART], lower limit of quantification [LLOQ]: 12 IU/ml) or Roche COBAS AmpliPrep/COBAS TaqMan assay (LLOQ: 15 IU/ml).

Results: At present 51 patients reached end-of-treatment and 33 end of follow up. Overall SVR12 rate was 29/33 (88%; see table). 2 FCH-patients died at weeks 8 and 20, respectively. Two patients interrupted treatment before week 12; one was transplanted at week10, the other was hospitalized for hepatic encephalopathy;

treatment was stopped at week8, but the patient reached SVR12. No episode of graft rejection was observed. Compared to baseline (BL) in SVR12-patients transaminases (BL/SVR12: ALT: 139±94.3 *vs.* 44±43.2; AST:133±105 *vs.* 44±48, $p < 0.001$) decreased, platelets (133±72.3 *vs.* 177±77.9 G/L [p = 0.037]), and serum-albumin (34.5±12.3 *vs.* 12:40.6±5.6 g/L [p = 0.048]) increased.

	Week 4 (68 evaluated [100%])		Week 12 (57 evaluated [83.8%])		SVR12 (33 evaluated [49%])
	TnD, n (%)	<LLOQ, n (%)	TnD, n (%)	<LLOQ, n (%)	n (%)
SOF/DCV [a]	12 (67%)	10 (43%)	22 (47%)	5 (71%)	14 (100%)
SOF/RBV	4 (22%)	7 (30%)	15 (32%)	1 (14%)	8 (89%)
SOF/SIM	2 (11%)	2 (9%)	6 (13%)	1 (14%)	4 (80%)
SOF/LDV [b]	–	3 (13%)	3 (6%)	–	3 (100%)
SIM/DAC	–	1 (4%)	1 (2%)	–	0 – relapse
Total	18 (26.5%)	23 (33.8%)	47 (83%)	7 (12%)	29 (88%)

EoT, end of treatment; SVR12, sustained virological response/week 12 follow-up; TnD, target not detected.
[a] + RBV in 1 patient.
[b] + RBV.

Conclusions: Real life-data of IFN-free DAA-based regimens in OLT-patients with HCV-recurrence show high on-treatment and SVR-rates with favorable safety-profile. Additionally, patients with successful treatment experienced improvement of graft function.

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IMPLICATIONS OF BASELINE HCV RNA LEVEL AND INTRAPATIENT VIRAL LOAD VARIABILITY ON OBV/PTV/R + DSV 12-WEEK TREATMENT OUTCOMES

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Introduction: High levels of pre-treatment HCV RNA may impact the risk of virologic relapse post-treatment. Within the ombitasvir/paritaprevir*/ritonavir and dasabuvir (3D) development program, we examined the effect of viral load on the risk of virologic relapse within various HCV RNA strata.
Material and Methods: Non-cirrhotic treatment-naïve HCV-infected patients who received 12 weeks of 3D (GT1b) or 3D+RBV (GT1a) were included in the analysis. Post-treatment relapse rates were summarized by pre-treatment HCV RNA thresholds. Intrapatient HCV RNA measurement variability was assessed by evaluating differences in HCV RNA levels between screening and baseline (median interval=3 weeks). Plasma samples were analyzed at a central laboratory using the Roche COBAS® TaqMan® RT-PCR assay v2.0.
Results: Among 618 patients, median baseline HCV RNA was 6.56 $\log_{10}$ IU/mL (3.6 million [M] IU/mL); 7/618 (1.1%) had post-treatment relapse. There was no association between baseline HCV RNA and relapse rate for any threshold (Table), with relapse rates of 1.2% and 0.9% below and above baseline HCV RNA of 10 million IU/mL, respectively. In patients who achieved SVR12 or relapsed, the median baseline HCV RNA was 6.55 and 6.66 $\log_{10}$ IU/mL, respectively (p = 0.2). No relapses were observed for any patient with viral load <2.5M IU/mL. Intrapatient variability in HCV RNA measurements increased with rising baseline viremia. Screening and baseline HCV RNA measurements differed by >1M IU/mL in 55%, by >2M IU/mL in 35%, and by >3M IU/mL in 26% of patients. In the subset of patients with a screening HCV RNA above 2M IU/mL, 79% differed by >1M IU/mL, 53% differed by >2M IU/mL, and 40% differed by >3M IU/mL. At the 6M IU/mL threshold, 18% of patients had discordant baseline and screening HCV RNA values.

Table: Relapse rates according to baseline HCV RNA thresholds chosen based on recent FDA analysis

Threshold	Below threshold	Above threshold
1 million IU/mL	0/157 (0%)	7/461 (1.5%)
1.5 million IU/mL	0/201 (0%)	7/417 (1.7%)
2 million IU/mL	0/223 (0%)	7/395 (1.8%)
2.5 million IU/mL	0/248 (0%)	7/370 (1.9%)
3 million IU/mL	1/269 (0.4%)	6/349 (1.9%)
3.5 million IU/mL	1/302 (0.3%)	6/316 (1.7%)
4 million IU/mL	2/324 (0.6%)	5/294 (1.2%)
5 million IU/mL	4/373 (1.1%)	3/245 (1.2%)
6 million IU/mL	4/410 (1.0%)	3/208 (1.4%)
7 million IU/mL	5/443 (1.1%)	2/175 (1.1%)
8 million IU/mL	5/467 (1.1%)	2/151 (1.3%)
9 million IU/mL	5/489 (1.0%)	2/129 (0.9%)
10 million IU/mL	6/502 (1.2%)	1/116 (0.9%)
Total	7/618 (1.1%)	

Conclusions: With this multi-targeted regimen, we did not identify any viral threshold for risk of relapse, suggesting that 12 weeks of therapy is optimal for minimizing the risk of relapse in naïve, non-cirrhotic patients, regardless of underlying host or viral factors. Intrapatient variability in HCV RNA measurements was common, suggesting that a subset of patients may be misclassified if viral thresholds are important for clinical decision-making.
*Paritaprevir was identified by AbbVie and Enanta.

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SB 9200, A NOVEL IMMUNOMODULATOR FOR PATIENTS WITH VIRAL HEPATITIS: PHASE 1 MAD STUDY IN PATIENTS WITH HEPATITIS C VIRUS (HCV) INFECTION

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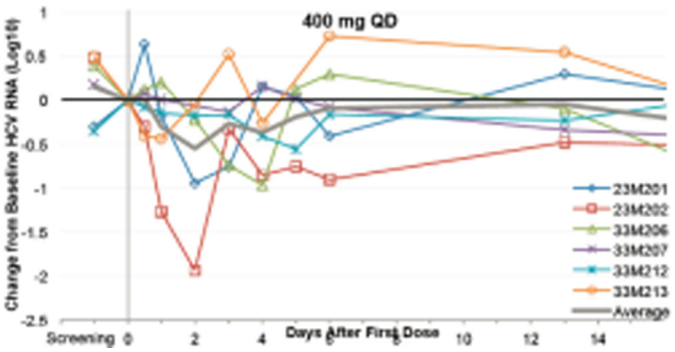
Introduction: The immune response has an important role in the clearance of HCV even in the era of highly potent direct acting antivirals (DAA's) particularly as shorter durations of therapy are explored. SB 9200 is a novel agonist of innate immunity that activates intracellular RIG-1 and NOD2, the cytosolic viral sensors that are important for the regulation of the innate immune response and activation of intracellular interferon (IFN) signaling pathways. SB 9200, a pangenotypic anti-HCV agent, is an oral dinucleotide prodrug that is enzymatically converted to the active Rp-, Sp-SB 9000 isomers in vivo.
Material and Methods: This was a first in man randomized, placebo-controlled, multiple ascending dose study of SB 9200 in treatment-naïve HCV infected adults. Subjects were randomized 6:2 to SB 9200 or placebo for 7 days. Doses evaluated were: 200 mg (N = 8), 400 mg (N = 8), 900 mg (N = 8 HCV-1, N = 6 HCV-3).
Results: Increases in SB 9200 AUC0-t and Cmax were dose-proportional. The terminal plasma half-life (t1/2) on Days 1 and 7 ranged from 0.684 to 1.07 hours for SB 9200, and was slightly longer for the metabolites: Sp-SB 9000 t1/2 was 4.65–8.90 hours, Rp-SB 9000 t1/2 was 4.31–5.94 hours.
SB 9200 Cmax ranged from 0.531 to 6.66 ng/mL at 0.817 and 3.00 hours. Sp-SB 9000 Cmax ranged from 7.13 to 22.0 ng/mL at 1.00 and 12.0 hours. Rp-SB 9000 Cmax ranged from 4.32 to 14.7 ng/mL at 1.00 and 12.0 hours.

Peak individual viral load drop improved from 1.5 to 1.9 $\log_{10}$ when the dose increased from 200 to 400 mg (Figure). Further dose increases did not result in response increases. Inter-individual variability in antiviral response was observed. A significant relationship between SB 9200 Cmax and maximum suppression of HCV RNA on Day 7 was observed (p = 0.015) after exclusion of two subjects with extreme Cmax values for SB 9200.

73 adverse events (AEs) were reported by 25 subjects (83.3%), mostly mild in severity. No dose limiting toxicities or systemic interferon-like side effects were observed and no serious adverse events were reported. There was no relationship between incidences, severity or relationship of AEs and dose of SB 9200 received or placebo.



Conclusions: SB 9200 is a novel oral agonist of innate immunity which achieves therapeutic concentrations of the drug upon once daily administration. Results of this study suggest an anti-viral effect similar to IFN, but without systemic side effects. SB9200 is a novel anti-HCV agent that merits further evaluation in combination trials with DAAs.

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LIVER FIBROSIS EPIDEMIOLOGY REVISITED USING SOFTWARE COMBINED BIOMARKER: PROOF OF CONCEPT USING 1,081,658 CENTRALIZED FIBROTEST (FT) PRESCRIPTIONS

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Introduction: The epidemiology of liver fibrosis (LF) was classically described by liver biopsy (LB), therefore limited by bias of LB indication and sample size. Several LF biomarkers have been validated including FT (AIM 2013). We aimed to revisit the epidemiology of LF using the centralized software-combined database (DB) of FT, for assessing the impact of age and sex on LF severity (LFS) as well as the impact of health policy for screening LFS.

Material and Methods: From 2002 to 2014, 1,081,658 interpretable FT, were performed consecutively on fresh serum, mostly for chronic hepatitis C (CHC). In 5 countries, France (Fr), USA, Romania (Ro), Egypt (Eg) and Morocco (Ma), we compare (R software): LFS (METAVIR scoring system F0 to F4 and cirrhosis severity F4.1 compensated, F4.2 bleeding-risk and F4.3 complicated, J Hepatol 2014), the density of LFS according to birth-year (BY) and sex, and the progression of FT dissemination. We used as controls a DB of CHC with LB (Lancet 1997 n = 2235, Gastro 2002 n = 4177).

Results: F4 prevalence varied from 13.8% (Ro), 14.9% (Fr), 18.1% (USA), 22.1% (Ma) to 28.2% (Eg). In France, FT was commonly prescribed, with stable rates since reimbursement (2006); a high number of F4 was detected in women born before 1945 (n = 20,672). In USA, FT (FibroSure) rate dramatically increased in 2013–2014 when the baby-boomer campaign (BY 1945–1965) started, vs 2011–2012 (+132%). In multivariate regression analysis, adjusted for age (OR = 1.06) and sex (0R=3.22) and USA as reference (OR = 1), Eg had the higher risk (OR = 2.3;2.2–2.3) possibly due to associated schistosomiasis; then Ma (OR = 1.3;1.2–1.3) and Ro (1.1;1.1–1.2; masked in univariate (younger age) and lower risk for Fr (0.90; 0.88–0.91); All $P < 10^5$. Sensitivity analyses (excluding age-sex-FT standardization, repeated FT, non-CHC) observed similar results. Associations between LFS age and sex were similar in LB-DB.

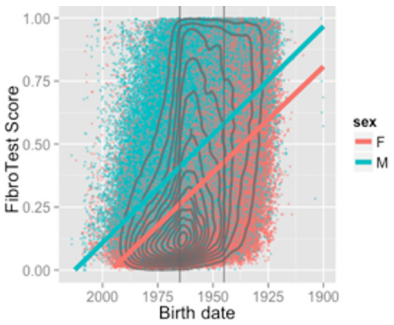

Figure: Density of LFS, according to BY and sex, in Fr (n = 513,762), USA (263,422), Ro (49,812), Eg (34,168) and Ma (16,542). Blue (men) and red lines (women) were regression lines LF/age; vertical black lines were 1945–1965 cutoffs, concentric black lines were density-10percentiles.

Conclusions: Despite possible bias associated with patented biomarker prescription, this proof-of-concept study permitted already to prepare better strategies to reduce the burden of cirrhosis, based on 200 times more patients than biopsy. The first interpretation for France is to prevent without delay a massive burden of death in women born before 1945.

LP42

SAFETY AND EFFICACY OF NOVEL ANTIVIRALS IN KIDNEY TRANSPLANT RECIPIENTS WITH CHRONIC HEPATITIS C VIRUS (HCV) INFECTION

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Introduction: Interferon and ribavirin are the mainstay therapy for HCV infection in the post-renal transplant (tx) population. Low treatment efficacy, side effects and concern for interferon-induced acute rejection have limited their use. Several oral direct-acting antivirals (DAA) were recently approved for HCV therapy and have demonstrated high efficacy and good safety profile in the non-tx population. To date, there are no studies evaluating the safety, efficacy, or viral kinetics of DAAs in the renal tx population, thus we sought to characterize our experience with DAA therapy in renal tx recipients.

Material and Methods: Retrospective chart review was conducted on post-renal tx pts with HCV who initiated DAAs between 12/2013–10/2014 at 3 sites. Demographics, clinical information and laboratory values were collected. The primary end point was sustained virologic response (SVR) at 12 wks post-treatment (Rx) (SVR12).

Results: Fifteen pts completed DAA therapy. Mean age was 55.8 yrs (range 34–70 yrs); 80% were male; 53% White, 20% Hispanic, 14% Asian and 13% Black. Mean BMI was 27 (range 21.5–45.5); 73% had diabetes and 87% had hypertension. Genotype distribution: 1a (67%); 1b (13%); 1 without subtype (13%); 2 (7%). Seven pts were Rx naïve; 4 were non-responders; 3 were relapsers; 1

Table 1 (abstract LP42). HCV viral loads and laboratory values at the various treatment time points

	Baseline	Week 4	Week 12	12 weeks post-Rx	p-value [a]
HCV RNA	3.35 million IU/mL (mean) 1060 to 19 million IU/mL (range)	Non-detected: 6 pts (46%) Detected: 7 pts (54%)	Non-detected: 12 pts (100%) Detected: 0 pt	Non-detected: 10 pts (83%) Detected: 2 pts (17%)	
Creatinine (mg/dL), mean (range)	1.26 (0.81–1.76)	1.41 (0.87–2.0)	1.34 (0.98–1.80)	1.40 (0.99–1.99)	0.07
ALT (U/L), mean (range)	86 (14–416)	27 (7–79)	21 (9–58)	31 (7–147)	0.02
Total bilirubin (mg/dL), mean (range)	0.78 (0.30–1.5)	1.05 (0.20–3.5)	0.70 (0.20–1.3)	1.21 (0.20–7.6)	0.5

[a] Calculated based on the comparison between the baseline and 12 weeks post-Rx values.

discontinued Rx. Six pts (40%) had cirrhosis and two (33%) had hepatic decompensation. Nine had kidney tx, 4 pts had combined kidney/liver tx and 2 pts had kidney/pancreas tx. DAA regimens included sofosbuvir (SOF)/simeprevir (SMV) in 9 pts, SOF/SMV/Ribavirin (RBV) in 3 pts, SOF/RBV in 2 pts and SOF/ledipasvir in 1 pt. All were treated for 12 wks. At the time of data analysis, 12 pts (100%) had undetectable end-of-treatment HCV RNA; 10 pts (83%) achieved SVR12 and 2 pts relapsed. The 2 relapsers were black, genotype 1a, had advanced fibrosis/cirrhosis, and received SOF/SMV. Four pts (27%) had potential Rx related side-effects: gout flare, diarrhea, photosensitivity and foot/calf pain. None led to Rx discontinuation. Two pts had worsening proteinuria awaiting work-up. Mean ALT was lower at post-Rx wk 12 and creatinine was not significantly different (Table 1). Calcineurin inhibitor trough levels were stable in all pts and there was no observed allograft rejection.

Conclusions: This is the first reported series of DAA use in renal tx recipients. These agents were efficacious (SVR12 83%), safe and well tolerated in this population. Viral relapse is uncommon and was only observed in pts with HCV genotype 1a and advanced fibrosis/cirrhosis.

LP43

WHAT IS REQUIRED FOR CONTROL AND ELIMINATION OF HEPATITIS B GLOBALLY?

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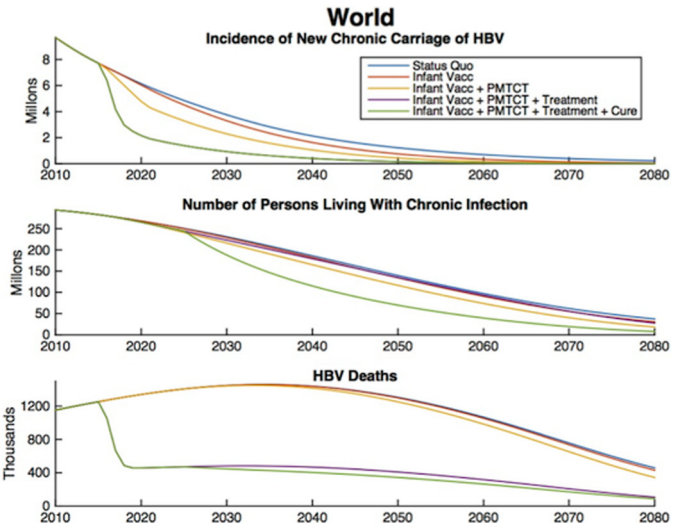
Introduction: Despite the availability of a safe and effective vaccine and treatment, variability of coverage means that HBV still accounts for up to one million deaths annually worldwide. Our aim was to evaluate the potential impact of scaling up interventions against HBV, set realistic targets for elimination of transmission and mortality due to HBV and identify key developments needed to achieve them.

Material and Methods: A dynamic, age, sex and region-structured mathematical model of the worldwide HBV epidemic was constructed incorporating data on epidemiology, vaccination coverage, treatment, regional demography and the natural history of HBV. The model was used to generate predictions regarding incidence of new chronic HBV infections, prevalence and HBV related mortality under assumptions that interventions remain at current levels ('status quo'). We then used the model to estimate the impact of scaling up of both prevention and treatment interventions, to establish what would be sufficient to bring HBV to the threshold of control and elimination by 2030.

Results: The scale-up of infant HBV vaccination to date, is already driving a decrease in new infections, and if maintained at current levels, will have averted 1.2M deaths by 2030. This will also result in a greater proportion of transmission being mother-to-child. Without further intervention, the number of persons living with HBV will remain at the same current high levels for the next 40–50 years and there will be 20M HBV-related deaths by 2030. However, a 90% reduction in incidence of new chronic infections and 65% reduction in mortality could be achieved by 2030 with the scale-up of existing interventions: >95% coverage of infant vaccination, 80% coverage of birth dose vaccine and 73% coverage of treatment for all those eligible (implemented from c. 2020). This would amount to 13M deaths averted worldwide by 2030, including 6M cases of cancer. The global cost is forecast to peak at $7.5B annually but decline rapidly after 2030, and could be accelerated if a cure is developed.



Conclusions: The fight against HBV through universal infant vaccination has scored major victories in reducing new infections in some settings but, without further intervention, prevalence and mortality will remain high for decades. However, expansion of vaccination and treatment could significantly reduce deaths and transmission. Although the costs are high, they peak at lower values and decline more rapidly than those projected for other infectious diseases with similar burden.

LP44

PHAGOCYTOSIS: HOW THE BEGINNING PROGRAMS THE END IN THE DAMAGED LIVER

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Introduction: Phagocytosis is a multi-step process that allows the elimination of harmful and dead cells. Professional phagocytes are immune cells, e.g. macrophages, endowed with the ability to process the cargo and present the antigens. Phagocytosis shapes the adaptive immune response and the local microenvironment, generating an anti-inflammatory milieu. The fine tuning of the anti-inflammatory process are not fully understood. In the fibrotic liver two major populations of macrophages exist: inflammatory and restorative macrophages. The formers are recruited at the onset of the injury and the latters mediates fibrosis remodeling later. Our hypothesis is that phagocytosis shapes the switch of the phenotype of inflammatory macrophages to restorative macrophages.

Material and Methods: We have performed *in vitro* and *in vivo* assays using a mouse model defective for the expression of Gpnmb, a protein involved in the formation of the phagosome. Macrophages

from Gpnmb$^-$ mice are able to ingest the cargo but not to process it. As *in vivo* model of liver fibrosis we have used the chronic administration of CCl_4. After CCl_4 withdraw mice experience a fibrotic phase dominated by inflammatory macrophages, followed by a phase of fibrosis remodelling characterised by restorative macrophages.

Results: Gpnmb$^-$ mice show a higher level of plasma transaminases in the phase of fibrosis remodelling, associated with higher proliferation of hepatocytes and higher levels of inflammatory cytokines in the tissue. Concomitantly, Gpnmb$^-$ mice show a lower number of macrophages and T cells. Flow cytometry analysis unveils a skew to a pro-inflammatory phenotype in the recovering liver but not in circulating monocytes. The role of IL6 as pro-proliferative to hepatocytes is established but its regulatory role on phagocytosis is unknown. Both *in vivo* and *in vitro* IL6 shows a pro-phagocytic effect and this effect seems to be mediated by the activation of the STAT3 pathway. After a single CCl4 injection the fraction of phagocytic macrophages in the liver of Gpnmb$^-$ mice increases from 25% to 85% when IL6 is co-administered ($n = 5$, $p < 0.01$, two-way ANOVA followed by Bonferroni's post-hoc test).

Conclusions: Our data suggest that phagocytosis could be the gatekeeper between the phase of initial inflammation and the phase of tissue remodelling and that IL6 could mediate this activity.

LP45

THE HEPATITIS B VIRUS (HBV) SURFACE ANTIGEN IMPEDES HEPADNAVIRAL REPLICATION-DEPENDENT INTERFERON RESPONSES IN A HBV TRANSGENIC MOUSE MODEL

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Introduction: Chronic hepatitis B virus (HBV) infection is an important cause of liver-related morbidity and mortality worldwide. Impairment of Toll-like receptor (TLR) signalling by the hepatitis B surface antigen (HBsAg) is supposed to attenuate immune responses, thereby facilitating chronicity of infection. Aim of this study was to characterize immune activation in different HBV transgenic mouse strains expressing or lacking the HBsAg.

Material and Methods: Mice expressing the HBsAg (Alb/HBs), mice replicating the complete viral genome but lacking HBsAg (Tg1.4HBV-S-Mut3) and the crossbreed strain (Alb/HBs x Tg1.4HBV-S-Mut3) expressing all viral proteins (HBV-s-rec) were analyzed. HBxAg-targeting small interfering RNAs (siRNAs) or TLR3 ligand polyinosinic-polycytidylic acid (Poly[I:C]) were injected intravenously into Tg1.4HBV-S-Mut3 mice, their HBV-negative littermates, and TLR3$^{-/-}$ and Tg1.4HBV-S-Mut3/TLR3$^{-/-}$ mice. Levels of immune-related genes, HBV DNA, HBcAg, HBeAg, and HBsAg were determined in primary liver cells, liver tissue and serum, respectively.

Results: Tg1.4HBV-S-Mut3 mice but not the HBsAg-recovered strain (HBV-s-rec) exhibited an increased and hepadnaviral replication-dependent expression of interferon beta (IFN-β), interferon-sensitive gene 15 (ISG15), and interferon-induced protein with tetratricopeptide repeats 1 (IFIT1). This antiviral response could be normalized by treatment with HBxAg-targeting siRNA and was completely abrogated in Tg1.4HBV-S-Mut3/TLR3$^{-/-}$ animals. Here, the lack of TLR3 signaling led to increased HBV replication. The HBV replication-induced TLR3 response was confined to non-parenchymal liver cells (NPC). Administration of Poly(I:C) further induced the expression of IFN-β, ISG15, and IFIT1 and thereby suppressed HBV replication *in vivo* and *in vitro*. However, induction of these antiviral genes was significantly lower in Tg1.4HBV-S-Mut3 mice than in control animals, a finding indicating HBs-independent immune evasion.

Conclusions: In contrast to HBV-infected patients mouse models for HBV infection show weak antiviral signals. The present data demonstrate that in the absence of HBsAg hepatic HBV replication leads to TLR3-dependent IFN responses mediated by NPC. We therefore hypothesize that HBsAg is the main evasion mechanism of HBV that controls antiviral innate responses in the liver.

LP46

OCCULT HBV IS HIGHLY PREVALENT IN PATIENTS WITH INTRAHEPATIC CHOLANGIOCARCINOMA AND IT IS DETECTED AS BOTH FREE EPISOMAL AND INTEGRATED DNA

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Introduction: Intrahepatic cholangiocarcinoma (ICC) is a fatal primary liver cancer with very poor prognosis. Genome-wide studies have made major advances in understanding the molecular basis of this disease, although most aspects remain unclear. Accumulating evidence indicates that chronic HBV infection is associated with an increased risk of ICC development and suggests an etiological role of HBV in the development of this tumor. Aims of the study were to investigate whether occult HBV infection (OBI) could be involved in ICC development and to characterize the molecular status of HBV in OBI-positive ICC specimens.

Material and Methods: Frozen paired tumor and non-tumor tissue specimens from 40 HBsAg-negative patients with ICC, who underwent surgical resection were tested for OBI by 4 different HBV-specific nested PCR. To reveal HBV cccDNA, DNA extracts were digested with a plasmid-safe ATP-dependent DNase and amplified by nested PCR with cccDNA-specific primers. Finally, for the detection of HBV DNA integrations the Alu-PCR technique was coupled to deep-sequence analysis.

Results: HBV genomic sequences were detected in tumor and/or non-tumor specimens from 28 of the 40 (70%) ICC patients analysed. In particular, 20/40 (50%) tumors and 13/23 (56.5%) non-tumor tissues were HBV DNA positive. HBV cccDNA was detected in tissue specimens from 10/28 OBI-positive patients (36%) (both in tumor and non-tumor specimens in 3 patients; only in tumor tissues in 4 patients; only in non-tumor tissues in 3 patients). HBV integrants were detected in 3 of 10 cases examined so far, and included portions of the HBx gene sequence (including the Basic Core Promoter/Enhancer II) in 2 cases and part of the core gene sequence in one case. The analysis of the integration sites revealed that the HBx sequences were located 3,374 nucleotides upstream the sequence encoding the cat eye syndrome critical region protein 5 isoform and within the coding sequence of the thromboxane A synthase 1, respectively, and that the core gene sequence was located within the cystinosin isoform 1 precursor coding sequence.

Conclusions: Occult HBV infection is highly prevalent in patients with ICC. Both free viral genomes and integrated DNA can be

detected in these cases. These results support the hypothesis of an involvement of HBV in the carcinogenic processes leading to ICC development.

LP47
EFFICACY OF TENOFOVIR DISOPROXIL FUMARATE TO PREVENT VERTICAL TRANSMISSION IN MOTHERS WITH LAMIVUDINE-RESISTANT HBV

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Introduction: In China, many chronically HBV-infected women of childbearing age receive lamivudine antiviral therapy at early ages. Thus, viral resistance becomes a challenge for intervention to prevent mother-to-infant transmission. We prospectively assessed the efficacy of tenofovir in pregnant women with lamivudine-resistant HBV.
Material and Methods: Chronic HBV-infected mothers resistant to lamivudine were enrolled. Tenofovir was administrated beginning at gestation week 24 or 28. HBV load and titers of HBsAg and HBeAg were ascertained every 4 weeks until delivery. All infants received standard combined immunoprophylaxis after birth and were followed for up to 1 year.
Results: Forty-eight mothers and their babies completed the treatment and follow-up, including 21 starting tenofovir therapy at gestation week 24 and 27 starting at week 28. Tenofovir treatment resulted in a mean HBV DNA decline of 5.23 ± 1.68 $\log_{10}$ IU/ml at delivery. Although the group starting therapy at week 24 exhibited more rapid viral inhibition, all mothers had a viral titer $<10^6$ IU/ml, and 73% (35/48) displayed virus <4 $\log_{10}$ IU/ml at delivery, a safe threshold for preventing HBV vertical transmission with combination immunoprophylaxis. No significant difference was found in the percentage of mothers having virus titers <4 $\log_{10}$ IU/ml between the two groups. Congenital abnormalities and neonatal growth were comparable to the normal population. No case of perinatal transmission was diagnosed.
Conclusions: This investigation clarifies the efficacy of tenofovir for blocking vertical transmission of HBV in mothers with lamivudine-resistant HBV strains and demonstrates that tenofovir is well tolerated in the second and third trimesters.

LP48
SVR12 RESULTS AFTER 12-WEEKS BOCEPREVIR, PEGINTERFERON AND RIBAVIRIN IN THE DUTCH ACUTE HEPATITIS C IN HIV STUDY (DAHHS)

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Introduction: The international epidemic of sexually transmitted acute hepatitis C virus (AHCV) continues to spread within HIV+ men having sex with men (MSM). The incidence rates reported vary between 0.08 and 1.75%. In HIV+ patients with acute HCV, relatively high cure rates (60–70%) are achieved with 24 weeks of peginterferon (P)]+/− ribavirin (R)]. Currently, none of the P-free regimens have been studied or registered for the treatment of AHCV. Furthermore, in most European countries and the USA, the availability of these regimens is restricted to patients with severe fibrosis/cirrhosis. Therefore, if the addition of a direct-acting anti-viral drug (DAA) to P would allow for a higher cure rate or shorter treatment duration, this would still be very valuable. In the DAHH-Study, the efficacy and tolerability of a 12-week boceprevir, P+R regimen in AHCV genotype-1 was evaluated in 10 Dutch HIV treatment centers.
Material and Methods: HIV+ patients with a new ALAT elevation were screened for the presence of HCV RNA. If positive, stored historical plasma samples were tested to prove that the HCV infection was recent. Boceprevir, P+R for 12 weeks was started without a P+R lead-in and was initiated no later than 26 weeks after the day of infection. Primary endpoint is sustained viral response at week 12 (SVR12) in patients with no HCV RNA detected (Roche, CAP/CTM target not detected) at w4 (RVR4) and in all patients included (secondary endpoint).
Results: From 9/2013 to 1/2015 we screened 127 HIV+ patients with a new HCV infection. We excluded 62 patients because of genotype 4 (n = 22), HCV infection >6 months (n = 17), spontaneous clearance before inclusion (n = 8), refusal to participate (n = 13) or other reasons (n = 2). 65 patients were included, 8 cleared HCV or refused before treatment initiation and 57 started therapy. RVR4 was 41/57 (72%) and end of treatment response was 41/45 (91%). In the RVR4 population, 22/23 (96%, 95% CI 76%<>99%) had an SVR12. In the ITT population SVR12 was 26/34 (76%, 95% CI 58%<>89%). Il28B genotype had no impact on RVR4 nor SVR12 results. SVR4 results of all patients and SVR12 results of 44 patients will be presented.
Conclusions: In HIV+ patients with an acute HCV infection, the addition of boceprevir to P+R cured 76% of the patients and as much as 96% of the RVR4 population. At 13,000 €, this shorter therapy is relatively cheap, reasonably tolerated and therefore a relevant therapy to prevent not only future liver disease but also ongoing transmission of HCV in HIV+ MSM.

LP49
SOFOSBUVIR CONTAINING REGIMES TO PATIENTS WITH HCV GENOTYPE 3 INFECTION. A SCANDINAVIAN REAL-LIFE EXPERIENCE

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Introduction: In registration trials the effect of sofosbuvir (SOF) containing regimes have been modest among patients with genotype 3 infection. Real life data are scarce in this group. We therefore aimed at assessing the effect of SOF containing regimes in genotype 3 patients seen in our daily practices.
Material and Methods: Consecutive patients were included at 8 treatment centers in Denmark, Sweden and Norway if they had genotype 3 infection, had received a SOF containing treatment regime and had at least 4 weeks of follow-up after end of treatment. The following treatments were administered according to local guidelines and availability of drugs: (1) SOF + ribavirin (RBV) for 24 weeks, (2) SOF + Daclatasvir (DCV) +/− RBV for 12 weeks or (3) SOF +pegIFN + RBV for 12 weeks. The

main endpoint was sustained virological response assessed at week 4 (SVR4).
Results: We included 82 patients with genotype 3. The mean age was 55 years (range 20–79), 55/67 (82%) with available staging were cirrhotic and 51% were treatment experienced.
SVR4 was achieved in 62 (83%) while 9 (11%) experienced virological relapse, 4 (5%) non-response and 1 (1%) viral breakthrough. Among 55 cirrhotics SVR 4 was achieved in 44 (80%) compared to 10/11 (91%) of non-cirrhotics. SVR 4 was obtained in 26/33 (81%) who received SOF+RBV for 24 weeks, 19/24 (79%) who received SOF+DCV +/− RBV for 12 weeks and 23/25 (92%) of those treated with SOF+pegIFN+RBV for 12 weeks. Among the 55 patients with cirrhosis SVR4 was achived in 15/22 (68%) who received SOF+RBV for 24 weeks, 16/19 (84%) who received SOF+DCV +/− RBV for 12 weeks and 16/18 (89%) who received pegIFN+SOF+RBV for 12 weeks. Among 50 patients with liverelasticity assessed 10/11 (91%) with <12.5 kPa achieved SVR compared to 14/15 (93%) with 12.5–25 kPa and 17/24 (71%) with >25 kPa.
Conclusions: SOF containing regimes to patients with genotype 3 infection induced SVR 4 in 83%.

LP51
TREATMENT OF SEVERE HCV RECURRENCE AFTER LIVER TRANSPLANTATION WITH SOFOSBUVIR BASED REGIMEN: RESULTS OF THE AISF-SOFOLT ITALIAN COMPASSIONATE USE PROGRAM

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Introduction: Hepatitis C virus (HCV) recurrence after liver transplantation (LT) can be associated with a rapid progression to cirrhosis, graft loss and/or death. Sofosbuvir (SOF) has been recently available in a compassionate use program for the treatment of severe HCV recurrence. The aim of this study was to evaluate the efficacy and impact on liver function of SOF based regimen in this setting.
Material and Methods: LT recipients with end stage liver disease (ESLD) or fibrosing cholestatic hepatitis (FCH) and estimated life expectancy ≤6 months were enrolled in a cohort study involving 16 Italian hepatology centers. All patients received 24 weeks of SOF (400 mg daily) in combination with pegylated interferon (PegIFN) and ribavirin (RBV) or an interferon-free regimen. The primary endpoint was the sustained virological response 12 weeks after treatment (SVR12).
Results: Of the 73 patients enrolled (49 ESLD and 24 FCH), 54 (74%) were males, 57 (78.1%) had genotype 1 infection and 46 (63%) had been previously treated. Median baseline MELD and Child–Pugh scores were 15 and 8, respectively. Fifty-nine patients (80.8%) received an interferon-free regimen (54 SOF+RBV, 4 SOF+Daclatasvir, 1 SOF+Simeprevir). To date, 69/73 had completed the study. Of them, 6 patients (8.7%) died (4 during the treatment and 2 during follow-up) and 1 underwent re-LT at week 16 of treatment, all treated with SOF+RBV. 18/69 (26.1%) had a virological failure: 1 breakthrough (treated with SOF+RBV) and 17 relapse (2/14 treated with SOF+PegIFN+RBV and 15/50 with SOF+RBV). SVR12 was achieved in 44 (63.8%): 28/45 (62.2%) in ESLD and 16/24 (66.7%) in FCH. 27/50 patients (54%) receiving SOF+RBV, 12/14 (85.7%) receiving SOF+PegIFN+RBV and 5/5 (100%) receiving SOF+Daclatasvir or Simeprevir obtained SVR12. RBV dose reduction and/or use of erythropoietin and/or blood transfusion due to anaemia were reported in 49.3% of cases. In patients with SVR12, an improvement of liver function was observed (Table). Median baseline MELD and Child–Pugh scores resulted higher in deceased than in survivors (26 vs 15, p = 0.013 and 11 vs 8, p = 0.006, respectively).

	Baseline	Week 12 post-treatment	P
MELD score	13 (7–32)	10 (6–20)	<0.001
Child–Pugh score	7 (5–15)	5 (5–9)	<0.001
INR	1.2 (0.9–4.5)	1.2 (0.9–1.7)	<0.001
Total bilirubin (mg/dl)	2.6 (0.5–12.4)	1.2 (0.4–5.8)	<0.001
Albumin (g/dl)	3.5 (2.4–4.7)	4 (2.7–5.2)	<0.001

Values expressed as median (range).

Conclusions: Treatment schedules with SOF in combination with PegIFN+RBV or a direct-acting antiviral agent provide high virological response rates in patients with severe HCV recurrence after LT. Viral eradication is associated with a significant improvement in liver function. Nevertheless, antiviral treatment seems not beneficial in subjects with very advanced disease.

LP52
NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) AS POTENTIAL RISK FACTOR OF CARDIOVASCULAR DISEASE AND ONCOLOGICAL DISEASE IN DIABETIC TYPE 2 PATIENTS

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Introduction: NAFLD is an increasingly cause of liver damage in western countries associated with obesity, hypercaloric diet and the sedentary lifestyle. The increasingly high prevalence of Nafld and its possible damage on several organs due to its inflammatory effects (cardiovascular risk and oncological risk) will lead to a prioritary health care problem in the next future. Validated prognostic scores for NAFLD and for cardiovascular risk in diabetics patients were respectively Fatty liver index (FLI) and UK Prospective Diabetes Study (UKPDS risk engine)
Material and Methods: The aims of our study are to assess the real correlation between FLI and UKPDS risk with cardiovascular (CE) and oncological events (OE) in a cohort of diabetic type 2 patients, in order to identify with accurancy the best predictor.
2004 patients referred to our Diabetics Center Ambulatory and in a regular follow-up were retrospectively tested. UKPDS risk and FLI were calcolated for each patient. Data such as CE, OE, anthropometric, biochemical and metabolic features were also

collected. T test for unpaired data and Pearson Chi-squared test were performed.

Results: 304/2004 pt (15%), 211 M and 93 F, were FLI >60; in this group we observed 14 (5%) OE (7 M and 7 F) and 81 (27%) CE (64 M and 17 F). 743/2004 pt (37%), 638 M and 17 F), were FLI <20; in this other group we observed 9 (1%) OE (6 M and 3 F) and 74 (10%) CE (47 M and 27 F). The statistical analysis showed that patients with FLI >60 have a higher risk of OE ($p = 0.0006$) or CE ($p = 0.0001$) compared to patients FLI <20. We identified also two peculiar profiles of cardiovascular risk, in fact male gender patients with FLI >60 presented a significant higher risk of developing CE than female ($p < 0.05$); instead female gender patients with FLI <20 presented a significant higher risk of developing CE than male ($p < 0.001$). No statistical significance was found between FLI >60 + UKPDS >20 and CE ($p = 0.754$). FLI >60 and FLI <20 patients also significantly differed respectively for mean age 62.2 vs 68.4 y ($p = 0.02$), duration of diabetes 4.9 y vs 13.24 y ($p = 0.002$) and mean glycated hemoglobin 8.7 y vs 7.9 ($p = 0.009$).

Conclusions: An early and aggressive program of follow-up and treatment could be established in diabetic type 2 patients with FLI >60 and so with reasonable suspicion of NAFLD because this population have higher risk to develop CE and OE in comparison to FLI <20 (or FLI negative and not suspicion of NAFLD). The simultaneous UKPDS and FLI positivity doesen't improve accuracy in predicting CE.

LP53

CLINICAL PREDICTIVE VALUE OF INSULIN SECRETION CHARACTERISTICS AND ABNORMAL GLUCOSE METABOLISM OF NAFLD PATIENTS

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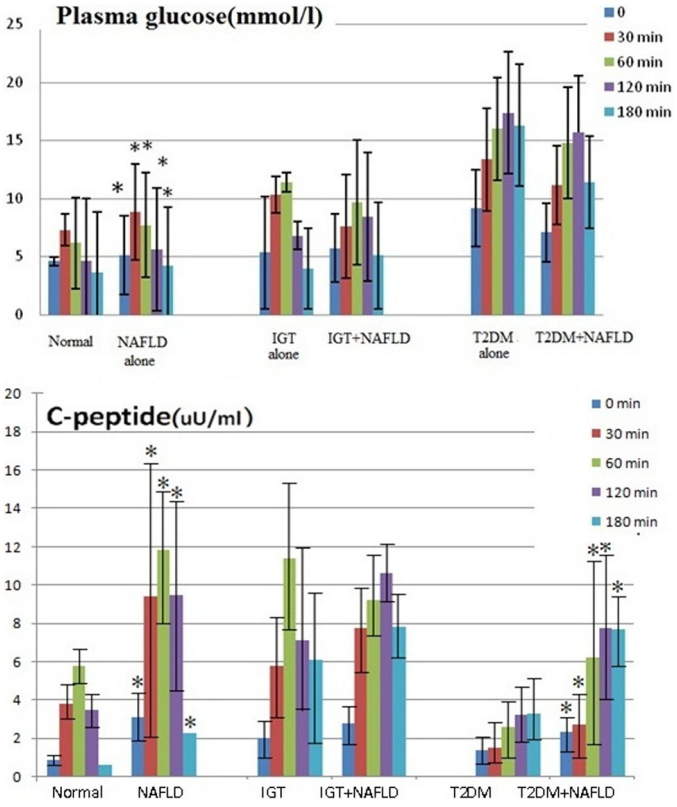
Introduction: Hyperinsulinemia is a common metabolic characteristic of NAFLD. However, the mechanisms of hyperinsulinemia remain to be fully elucidated. Nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) often coexist and have adverse outcomes.

Material and Methods: 97 subjects with diagnosed NAFLD by magnetic resonance spectroscopy and 77 Waist circumference and BMI-matched subject were studied. Patients took a 75-g oral glucose tolerance test (OGTT), which measured serum insulin and C-peptide (C-p) levels at baseline (0 min), 30 min, 60 min, and 120 min after glucose challenge. They were classified further as normoglycemia, IGT or T2DM subgroups based on American Diabetes Association criteria. Hepatic insulin clearance, sensitivity and beta cell function were calculated using homeostasis and postprandial models.

Results: β-cell hypersecretion was significantly in the fat liver groups, even fat liver with T2DM compared with those in T2DM alone (HOMA2-B% $P < 0.005$ and $\Delta I_{0-30}\Delta G_{0-30\,min}$ $P < 0.001$). Insulin sensitivity were markedly decreased in fat liver patients, compared to those of normal control subjects (Matsuda index $P < 0.001$ and HOMA2-S% $P < 0.0009$). Slightly, but not significantly, lower in fat liver subjects with IGT or T2DM than those in IGT or T2DM alone. Hepatic insulin clearance was not lower in fat liver patients than nonfat liver patients with normoglycemia and IGT or T2DM alone ($P > 0.05$). Linear regression analysis showed that $\Delta Glucose_{0-120\,min}$ was the best independent predictive variance ($P = 0.002$). $\Delta Glucose_{0-120\,min}$ appeared to be the best diagnostic capacity of NAFLD with a c-statistic 0.901 (95% CI 0.81–0.99) using ROC curve.



Conclusions: Hyperinsulinemia is primarily caused by β-cell hypersecretion in fat liver patients with normoglycemia and IGT, even with T2DM controlled by glycemia-matched subjects, but not reduced hepatic insulin clearance. That give us the impression that NAFLD is not the result of insulin resistance but the complimentary effect of glucose dysregulation. Glycemia indexes such as $\Delta Glucose_{0-120\,min}$ appeared to be the best model to predict the severity of NAFLD than liver injury indices ALT, AST and γGT. In patients with similar levels of insulin resistance and hyperglycemia, DM-NAFLD was associated with higher serum insulin levels than T2DM alone. The present study demonstrates pathophysiological differences in mechanisms of insulin resistance in patients with DM-NAFLD versus T2DM alone.

LP54

SOFOSBUVIR INHIBITS HEPATITIS E VIRUS REPLICATION IN VITRO AND RESULTS IN AN ADDITIVE EFFECT WHEN COMBINED WITH RIBAVIRIN

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Background and Aims: Infection with hepatitis E virus (HEV) of genotype 3 may result in chronic hepatitis in immunocompromised patients. Reduction of immunosuppression or treatment with ribavirin or interferon-α can achieve viral clearance. However, safer and more effective treatment options are needed.

Material and Methods: A selectable subgenomic HEV genotype 3 replicon was derived from the Kernow-C1 strain by partial substitution of the open reading frame 2 (ORF2) and ORF3 sequences with the neomycin phosphotransferase gene. A continuous Huh-7 human hepatocellular carcinoma-derived cell line harboring autonomously replicating HEV RNA was established by transfection of this replicon and selection with neomycin. The antiviral effect of sofosbuvir (SOF), a potent nucleotide inhibitor of the hepatitis C virus (HCV) polymerase, as well as interferon-α,

ribavirin (RBV) and nesbuvir, a non-nucleoside inhibitor of the HCV polymerase, were assessed by quantitative RT-PCR. Findings were validated in a transient luciferase-expressing HEV genotype 3 replication system as well as a full-length infectious clone of HEV. Cell viability was monitored in parallel.
Results: SOF inhibits HEV RNA replication in the selectable subgenomic replicon system, with an IC_{50} of 1.2 μM and without any measurable cell toxicity. As expected, interferon-α and RBV inhibited HEV RNA replication as well, with IC_{50} of 8.5 IU/ml and 8.0 μM, respectively, while nesbuvir as an allosteric inhibitor of the HCV polymerase did not inhibit HEV (negative control). Findings were confirmed in the transient replication system as well as the full-length infectious clone of HEV. Moreover, the combination of SOF and RBV resulted in an additive antiviral effect against HEV.
Conclusions: The HCV polymerase inhibitor SOF inhibits HEV replication and results in an additive effect when combined with RBV. These findings yield potential new avenues for the treatment of persistent HEV infection in immunocompromised patients with chronic hepatitis E.

LP55

RESPONSE GUIDED THERAPY IS NOT DEAD: LOW SUSTAINED VIROLOGIC RESPONSE (SVR) RATES IN PATIENTS WHO HAVE DETECTABLE HEPATITIS C VIRUS (HCV) AT WEEK 4 OF TREATMENT WITH SOFOSBUVIR (SOF) CONTAINING REGIMENS

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Introduction: Historically, HCV treatment has been response guided. Clinical trials data with SOF indicated on treatment virologic response was not predictive of SVR. With the introduction of SOF in the United States (US) treatment guidelines eliminated response guided therapy (RGT) that depended on on-treatment viral testing.
Material and Methods: We have a consortium of 6 centers in the US who treat HCV in specialty clinics and also run a Project ECHO (Extension for Community Healthcare Outcomes) program where, through longitudinal videoconferencing, case-based learning and mentoring, primary care clinicians are trained to treat HCV. Members of the consortium and their primary care partners follow shared HCV treatment guidelines. Because of concern that 4 week HCVRNA could be an important predictor of SVR in a real-world setting, we continued to measure on treatment 4 week HCVRNA. Data presented in this abstract are from patients treated either at the specialty clinic or by primary care clinicians through Project ECHO. The purpose is to examine the association between 4 week HCVRNA and SVR in patients treated in real-world practice.
Results: Data on 360 consecutive patients started on treatment between 1/1/2014 and 8/20/2014 with SOF-containing regimens (pegylated interferon + ribavirin (RBV) + SOF; RBV+SOF; SOF + simeprevir) were analyzed. Patients were age 23–80, 46.6% female, 50% had APRI score (AST to platelet ratio index) ≥1.0 and 28.1% had a detectable 4 week HCVRNA. Overall SVR rate was 81.9%. For those with a nondetectable 4 week HCVRNA, SVR was 85.3%. For those with a detectable 4 week HCVRNA, SVR was 73.3%. Detectable 4 week HCVRNA was strongly associated with failure to achieve SVR (Chi-Square 7.14, p-value 0.007). In multivariable logistic regression analysis, detectable 4 week HCVRNA was independently associated with virologic failure with an Odds Ratio of 1.95 (95% Confidence Limits 1.07–3.56). As might be expected, APRI ≥1 was also independently associated with virologic failure with an Odds Ratio of 2.75 (95% Confidence Limits 1.47–5.14).
Conclusions: In a real-world setting, a significant proportion of SOF treated patients have detectable 4 week HCVRNA on treatment. A detectable 4 week HCVRNA is associated with virologic failure. More data are needed to formulate guidance for RGT with newly available HCV therapies.

Liver transplantation/Surgery: a. Experimental

P0001

EARLY MONOCYTE DYSFUNCTION IN PATIENTS WHO DEVELOP SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) AND SEPSIS AFTER HEPATOPANCREATICOBILIARY SURGERY

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Background and Aims: Patients undergoing major hepatopancreaticobiliary (HPB) surgery are at risk of life-threatening complications including systemic inflammatory response syndrome (SIRS) and sepsis. Early identification of at-risk patients would allow tailored post-operative care and improve survival. Serum interleukin-6 (IL-6) levels are upregulated in patients with poor outcomes after surgery, but the mechanisms that underlie this are unknown. Our aim was to study the pathways involved in IL-6 production in patients undergoing HPB surgery with the goals of understanding mechanisms leading to enhanced inflammatory responses and identifying biomarkers of adverse clinical consequences.
Methods: Two separate cohorts of adult patients undergoing major liver or pancreatic surgery and who gave written informed consent were studied (combined n = 69). Bloods were taken pre-operatively, and on day 1 and day 2 post-operatively. Serum and peripheral blood mononuclear cells were separated and immune phenotype and function were assessed *ex vivo*.
Results: Innate immune dysfunction was evident in blood samples taken from 12 patients who subsequently developed SIRS (on post-operative day 6) compared to 27 who did not, when no clinical evidence of SIRS were apparent (pre-operatively and on post-operative days 1 and 2). In patients who developed SIRS, $CD14^+$ monocytes sampled on post-operative day 1 and 2 expressed higher levels of Toll-like receptor (TLR)4 and TLR5 and produced significantly more IL-6 in response to stimulation with bacterial ligands [lipopolysaccharide (LPS) and flagellin] for these receptors ($p < 0.0001$). TLR-driven phosphorylation of NF-κB was increased on day 1, and interferon alpha-mediated STAT1 phosphorylation was higher pre-operatively in patients who developed SIRS. Increased *TLR4* and *TLR5* gene expression in whole blood was demonstrated in a separate validation cohort of 30 patients undergoing similar surgery. Expression of TLR4/5 on monocytes, particularly intermediate $CD14^{++}CD16^+$ monocytes, on post-operative day 1 or day 2 predicted SIRS

development with accuracy 0.89–1.0 (areas under receiver operator curves).

Conclusions: These data demonstrate the mechanism for IL-6 overproduction in HPB patients who develop post-operative SIRS and identify markers that predict patients at risk of SIRS 5 days before onset of clinical signs.

P0002

LIVER REGENERATION IS NOT IMPACTED IN THE ABSENCE OF INTESTINAL MICROBIOTA

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Background and Aims: Liver regeneration after partial hepatectomy is a very complex process that involves a variety of different liver cell types via cytokine- and growth-factor-mediated pathways. Bacterial products and LPS circulate in the portal blood stream directly through the hepatic sinusoids and have been proposed to initiate liver regeneration by activation of parenchymal and non-parenchymal cells in liver after partial hepatectomy. Our aim was to evaluate liver regeneration in the absence of microbes and microbial products.

Methods: Two third partial hepatectomy (PH) was performed in germ-free (axenic) mice and mice colonized with altered schaedler flora (ASF) were used as controls. Surgery was performed in axenic conditions to maintain sterility throughout the course of the experiment. Liver regeneration, injury and hepatic fraction of immune cells were assessed in tissue and serum samples 48 hours post partial hepatectomy. Proteomic screen was performed in livers from ASF and axenic mice 2 hours after PH.

Results: Liver injury (ALT/AST) and survival was not significantly different between germ free and ASF colonized mice. Liver regeneration was measured by the number of Ki67 positive cells and there was no significant difference between the germ-free and ASF mice ($p = 0.84$). Absence of microbiota was proved by fluorescence microscopy and faecal cultures. Fractions of CD4 T cells, NKT cells and B cells were altered at baseline between germ-free and ASF mice, but not after partial hepatectomy. The analysis of various protein pathways reveal specific differences between axenic and ASF mice that are present under basal conditions as described before but also persist after PH.

Conclusions: Liver regeneration after PH is not impaired in the absence of microbes and microbial products. These findings contrast with previous studies and reveal that the gut microbiota is not an essential for the priming or maintenance of liver regeneration.

P0003

SEQUENTIAL EXPRESSION PROFILES OF CANNABINOID RECEPTORS ON IMMUNE CELLS AND RELEASE OF CYTOKINES DURING LIVER REGENERATION

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Background and Aims: The mammalian liver has unique property to regenerate upon tissue loss, which induces quiescent hepatocytes to enter the cell cycle and undergo limited replication under the control of multiple hormones, growth factors, and cytokines. Cannabinoid receptors are well known characterized with distinct physiological properties. Recent studies have shown that cannabinoid receptors are critical for the release of various cytokines like TNF-α and IL-6 and play an important role in liver regeneration. The present study aims to evaluate the liver regeneration in subjects undergoing right lobe donor hepatectomy for LDLT (human regenerative model) and to investigate the role of peripheral blood cannabinoid receptor expression on immune cells and its effect in stimulating circulating TNF-α and IL-6.

Methods: 25 consecutive healthy and asymptomatic donors undergoing right lobe donor hepatectomy for Living donor liver transplant were included in the study. The study was approved by Institute Review Board and Ethical Committee. Venous blood samples were taken before surgery (Day 0) and on Post Operative days (POD) 1, 3, 7, 14 and 42. PBMCs were isolated by ficoll gradient method and RNA was extracted. The cannabinoid receptors expression profile was evaluated by RT-PCR at days 0, 3 and 7, whereas TNF-α and IL-6 were measured in plasma at different time points by ELISA.

Results: Very low or faint expression of CB1 and CB2 were seen on PBMCs at day 0. Significant increased expression of CB1 was observed at POD 3 (15 fold) and 7 (30 fold) compared to Day 0. However, CB2 expression was first increased at day 3 (8 fold) and decreased at Day 7 (Figure 1). Significant rise in the TNF-α and IL-6 levels was observed only on POD 1 ($p = 0.03$; $p < 0.0001$) but not subsequently for TNF-α. The increased IL-6 levels on POD 3 ($p = 0.01$), 7 ($p = 0.003$), 14 ($p = 0.002$) and 42 ($p = 0.03$) remained higher than Day 0. This may indicate the presence of an active CB1 receptor on PBMCs and plasma secretion of IL-6 during regeneration.

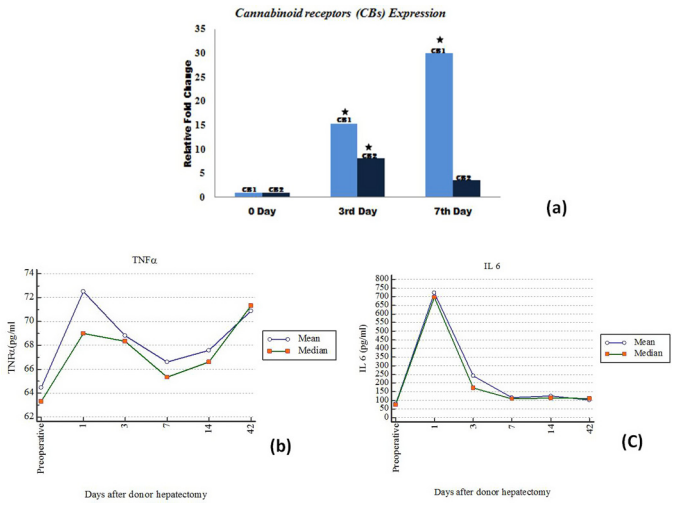

Figure 1.

Conclusions: The present study confirms the sequential expression of CB1 receptor which suggests its major role in liver regeneration in

donors undergoing partial hepatectomy. The novel findings reveal the release of cytokines TNF-α and IL-6 (regenerative markers) which might be influenced by CB1 receptor. This could pave way an insight to understand the role of cannabinoid receptors in liver regeneration.

P0004

THE TRANSPORTABLE MACHINE PERFUSION AIRDRIVE®, A NOVEL APPROACH TO SAFELY EXPAND THE DONOR POOL FOR LIVER TRANSPLANTATION

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Background and Aims: Livers derived from donation after circulatory death (DCD) suffer warm ischemia (WI) and are infrequently used for transplantation; they have the potential, however, to considerably expand the donor pool. The Airdrive® is the first transportable oxygenated machine perfusion (MP) unit suitable for liver preservation. We aimed to determine whether the Airdrive® MP would improve the quality of these livers using a DCD large animal model.
Methods: Female large white pigs were used as organ donors and recipients. Liver allografts procured from heart beating donors and preserved by simple cold storage (SCS) served as controls (n = 6). In experimental groups, cardiac arrest was induced by IV injection of KCL. After 60min WI, livers were perfused in situ with KTK and subsequently preserved either by SCS (SCS group, n = 3) or hypothermic MP (Airdrive® group, n = 4) using MPS-Belzer solution. After 4 hr of preservation, all livers were transplanted into recipient pigs. The main judgment criterion was animal survival at day 5
Results: All animals that received a simple cold stored liver allograft after 60min WI experienced PNF and died within 6 hours after transplantion (5-day survival = 0%). In contrast, 5-day survival in animals that received a MP liver was 100% (4/4, Airdrive® group) as well as in controls (6/6). A post-reperfusion syndrome was observed in all animals (3/3) of the SCS group but none of the control or Airdrive® groups. These phenomena caused a significant increase in fluid challenge and catecholamine needs in SCS group relative to control and Airdrive® groups. At the end of cold preservation, ATP content was significantly higher in the Airdrive® group vs. SCS group. After reperfusion, MP livers functionned better (albumin production, prothrombin time rates) and showed less hepatocellular and endothelial cell injury, in agreement with better preserved liver integrity (histology) vs. SCS group. MP livers also exhibited higher ATP reccovery than SCS livers. The protective effect of the Airdrive® device was associated with a significant attenuation of oxidative stress (lower lipid peroxidation, higher catalase and superoxyde dismutase activity), and a better endoplasmic reticulum adaptation leading to limit mitochondrial damage (cytochrome c, caspase 9, GLDH), and apoptosis (caspase 3 and TUNEL)
Conclusions: This study demonstrates for the first time the efficacy of the transportable MP Airdrive® device to enhance donor liver viability for transplantation in a clinically relevant DCD model.

P0005

THE NEW STRATEGY OF AUTOLOGOUS LIVER CELL TRANSPLANTATION FOR ACUTE LIVER FAILURE AFTER MASSIVE HEPATECTOMY: ROLE OF HEPATOCYTES AND LIVER NON PARENCHYMAL CELLS

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Background and Aims: Post-operative liver failure is one of the most critical complications of extensive hepatectomy. Allo-hepatocyte transplantation has been considered as an attractive therapy for lethal post-hepatectomy liver failure. On the other hand, resected livers of patients with hepato-biliary cancers contain not only cancer cells but also large amounts of normal hepatocytes. In this study, we aimed to utilize normal liver cells in resected livers, and evaluate the effect of autologous liver cell transplantation in rat models of post-hepatectomy liver failure.
Methods: Post-hepatectomy liver failure was induced by 90% partial hepatectomy (PH). The isolated graft liver cells which consisted of hepatocytes and non-parenchymal cells (NPCs) were transplanted into mesenteries of hepatectomized rats. We evaluated the effect of autologous liver cell transplantation on hepatectomized rats, the function of remnant livers and transplanted hepatocytes. Furthermore, in order to investigate which the hepatocytes or NPCs played an effective role in the autologous transplantation, the liver cells were separately divided into hepatocytes and NPCs, and transplanted, respectively.
Results: The survival rate of the liver cell transplantation group (69.2%) was significantly improved ($p = 0.00043$) in comparison to the control group (7.7%) 7 days after PH. The transplanted liver cells produced albumin in the NARs during 24 hours after transplantation. Although the viable transplanted cells deteriorated in the transplanted site, histological findings and adenosine-5′-triphosphate assay revealed the protective effect of transplanted cells on the remnant livers. The dual roles of the transplanted cells, one of them was the compensation of the proper hepatic function and the other was protection of the native liver would affect the rescue of fatal liver failure. And, hepatocytes could protect remnant livers and that NPCs could not protect remnant livers. This protective effect for remnant liver could lead to improve the survivorship in this liver failure model.
Conclusions: This study demonstrates the autologous liver cell transplantation could lead to the improvement of the survivorship. Our results indicated that we would get over donor shortage and rejection in the future.

P0006

HEPATIC ISCHEMIA/REPERFUSION INDUCES CHANGES IN TISSUE AND BILIARY LEVELS OF ARGININE AND ITS METHYLATED DERIVATIVES

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Background and Aims: Arginine (Arg), the substrate for nitric oxide synthase (NOS), can be methylated to form symmetrical dimethylarginine (SDMA) and asymmetrical dimethylarginine (ADMA), the last an endogenous inhibitor of NOS. SDMA is mostly excreted in the urine, while ADMA is mainly subjected to degradation in the liver. We recently reported that ADMA is also secreted in bile (Ferrigno et al., 2014). Arg competes with ADMA and SDMA for cellular transport across cationic amino-acid transporters (CATs) (Closs et al., 1997). Here we evaluate the changes in serum, tissue and biliary levels of Arg, citrulline (Cit), ADMA and SDMA as well as the modifications in CATs after ischemia-reperfusion (I/R) injury.
Methods: Male Wistar rats were subjected to 30-min partial hepatic ischemia by clamping the hepatic artery and the portal vein (n = 8) or sham-operated (n = 7). After 60-min reperfusion, serum, tissue and bile samples were collected and the concentrations of ADMA, SDMA, Arg and Cit were measured. AST, ALT and Alkaline Phosphatase (AP) levels in serum and bile were also determined. Using RT-PCR analysis, mRNA of cationic transporters, 2A (CAT-2A) and 2B (CAT-2B), for Arg and its methylated derivatives were also quantified.
Results: A decrease in serum Arg and an increase of Cit were detected after I/R; no changes in ADMA and SDMA were found. Both forms of methylarginenes, Arg and Cit were also detected in bile: a significant increase in ADMA and Cit and a decrease in SDMA and Arg were observed in bile at the end of reperfusion. On the contrary, lower ADMA levels and higher SDMA levels were quantified in liver biopsy after I/R when compared with sham-operated rats. A marked increase in AST, ALT and AP levels in serum and bile confirmed both hepatocyte and cholangiocyte damage. A decrease in mRNA transporter CAT-2A but not in CAT-2B was detected.
Conclusions: This study supported our previous results on the biliary ADMA clearance and demonstrated, for the first time, that the liver is also responsible for the biliary excretion of SDMA: the clearing of SDMA is not only confined to the kidney, but the liver could also take up SDMA from the portal and systemic circulation. These data also suggested that tissue and biliary changes in ADMA and SDMA should be CAT-2B transporter-dependent representing a step forward in the understanding of the mechanisms involved in the control of Arg and its methylated derivatives during hepatic I/R.
Supported by Fondazione Cariplo, grant no. 2011-0439.

P0007

HEPATIC T CELLS DERIVED FROM NORMOTHEMIC MACHINE PERFUSED GRAFTS CONTAINED MORE REGULATORY T CELLS AND FEWER PROINFLAMMATORY CYTOKINES PRODUCING T CELLS THAN THOSE FROM COLD STORAGE GRAFTS

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Background and Aims: In liver transplantation, ischemia reperfusion (I/R) injury is associated with an inflammatory response that has an impact on graft and patient outcomes. Ex-vivo normothermic machine perfusion (NMP) of donor allografts prior to transplantation has the potential to attenuate I/R injury, leading to improved liver function following implantation and increased utilization of grafts from marginal donors. Livers contain a significant amount of mononuclear cells that are involved in hepatic inflammatory processes. To assess the influence of NMP on hepatic inflammation, the phenotype and function of intra-hepatic lymphocytes derived from NMP- versus cold storage (CS)-preserved livers were compared.
Methods: All samples were obtained from liver allografts used for the phase I clinical trial of the Organanox NMP device. Second passage perfusates were collected from 12 grafts donated after brain death (DBD) immediately after NMP and compared to that of 21 CS grafts. Hepatic mononuclear cells (HMC) contained in the perfusate were isolated by density gradient centrifugation. Flow cytometry was used to define the phenotypes and the proportions of activated cytokine producing HMC by utilizing staining with various leukocyte lineage and activation markers.
Results: The mean preservation time was 11.5 hours for NMP and 9.5 hours for CS. Although the frequency of CD4+ T cells, NK and NKT cells were comparable between NMP and CS grafts, the frequency of CD8+ T cells ($p = 0.01$) and CD4+ CD25+ FOXP3+ regulatory T cells (Tregs) ($p = 0.03$) was significantly higher in NMP than in CS liver grafts. The proportions of both CD4+ and CD8+ T cells producing the pro-inflammatory cytokines IFNg ($p = 0.008$ and $p = 0.006$, respectively) and IL-17 ($p < 0.001$ and $p < 0.001$, respectively) was significantly lower in NMP than in CS grafts. Additionally, there were fewer CD4+ T cells producing the T cell trophic factor IL-2 in NMP versus CS allografts. There were no significant differences in T cell expression of immunosuppressive cytokines IL-10 or TGF-b.
Conclusions: These data demonstrate more Tregs and less pro-inflammatory T cells in NMP allografts, as compared to their CS counterparts. Brain death has been previously characterized as a pro-inflammatory state and the utilization of NMP rather than standard cold storage techniques modulates the allograft inflammatory response. Further studies are warranted to determine if these findings are associated with the improved clinical outcomes.

P0008

A MULTIDRUG STRATEGY TO ATTENUATE ISCHEMIA REPERFUSION INJURY IN EXPERIMENTAL LIVER TRANSPLANTATION

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Background and Aims: To develop a multidrug strategy designed to target various pathophysiological ischemia reperfusion injury (IRI) mechanisms in liver transplantation (LT), focusing on high-mobility group box 1 (HMGB1) release as a molecular marker.
Methods: Orthotropic liver transplantation with arterialization was performed in rats after 8 hours cold storage in HTK solution. Donors underwent i.v. multidrug preconditioning with glycine, taurine, alanine, arginine and prednisolone. Controls received the same volume normal saline i.v. prior to organ donation.
Results: Trypan blue staining and transmission electron microscopy revealed that necrotic cell death of sinusoidal endothelial cells and ischemic changing of hepatocytes were attenuated significantly after multidrug preconditioning. Further HMGB1 was decreased in rinsed solution compared to controls, preconditioning with glycine alone and after preconditioning with a combination of glycine and taurine ($P = 0.0308$). The examination at 1, 3 and 5 hours after reperfusion showed that multidrug preconditioning significantly decreased time-dependent necrotic changing and inflammatory response, and decreased the risk of early death after transplantation

(p = 0.0289) depicted by H&E histology, serum liver enzymes immunohistochemisty for HMGB1, IL1, IL6, TNFα and CD3 and CD68 positive cells.
Conclusions: Multidrug donor preconditioning protects liver grafts from injury during the process of transplantation. HMGB1 might be a molecular maker that predicts early graft survival.

P0009
THE EFFECT OF GLUTAMINE IN THE LIVER INJURY RESULTING FROM INTESTINAL ISCHEMIA AND REPERFUSION IN RATS
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Background and Aims: The intestinal ischemia-reperfusion (I/R-i) can cause cellular damage to the tissue and in distant organs such as the liver. Some aggressor agents are involved in these processes, such as: the generation of free radicals, the nitric oxide and the release of pro-inflammatory cytokines. Due to the involvement of free radicals in the lesions of I/R-i, some therapeutic antioxidants options are being studied and tested in I/R-i lesions. The aim of the study was to evaluate effects of glutamine (Gln) in an animal model of I/R-i.
Methods: Twenty male Wistar rats were divided into four groups: Sham operated **(SO)**, Glutamine + Sham operated **(G+SO)**, ischemia–reperfusion **(I/R)**, Glutamine + ischemia–reperfusion-i **(G+I/R)**. The rats were subjected to occlusion of the superior mesenteric artery for 30 min followed by 15 min of reperfusion. The glutamine (25 mg/kg/day) was administered 24 and 48 h before I/R. Systemic injuries were determined by evaluating liver segments for oxidative stress using lipid peroxidation (LPO), levels of nitric oxide metabolites (NO) and the expression of the enzyme inducible nitric oxide synthase (iNOS) by western blot analysis. The statistical analysis used was ANOVA followed by Student–Newman–Keuls (mean±SEM) significant at $p < 0.05$.
Results: The animals treated with glutamine showed a significant reduced the expression of iNOS compared to animals of I/R group – Liver (**SO:** 1.17±0.33; **G+SO:** 0.86±0.30; **I/R:** 2.51±0.10; **G+I/R:** 1.86±0.13). The LPO levels showed a significant reduced the animals treated with glutamine compared to animals of I/R group – Liver (**SO:** 0.16±0.01, **G+SO:** 0.20±0.02, **I/R:** 0.45±0.03; **G+I/R:** 0.24±0.02). The NO levels also showed a reduction in the treated animals compared to animals of I/R group – Liver (**SO:** 5.4±0.8; **G+SO:** 6.9±0.8; **I/R:** 15.6±2.7**;** 1.8±0.2; **G+I/R**: 6.1±1.1).
Conclusions: In conclusion, we suggest that pretreatment with Gln reduced oxidative damage and decreased levels of NO and iNOS expression in liver tissue of animals subjected to intestinal ischemia and reperfusion model.

P0010
FETAL HEPATOCYTE AS A CELL SOURCE OF LIVER TISSUE ENGINEERING USING A DECELLULARIZED MATRIX
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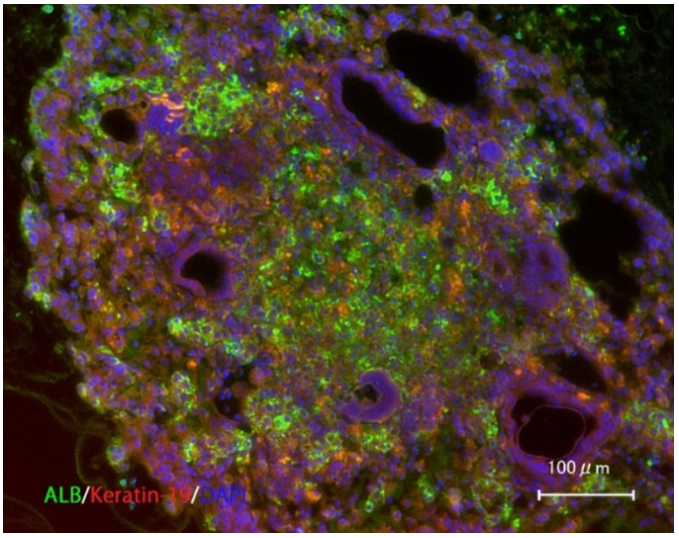
Background and Aims: The therapy of choice for end-stage liver disease is whole-organ liver transplantation, but this chance is limited by a shortage of donor organs. Cell-based therapies and hepatic tissue engineering have been studied as alternatives to liver transplantation, but neither has proven effective to date. A regenerative medicine approach for liver replacement has recently been described that includes the use of a three-dimensional organ scaffold prepared by decellularization of xenogeneic liver. Although the decellularized liver matrix has considered as an ideal scaffold of artificial liver, the strategy has not yet achieved a sufficient function up to expectation. The present study investigated the efficacy and potential of fetal hepatocyte as a cell source of liver tissue engineering using a decellularized matrix.
Methods: Mice fetal (E14.5) or adult hepatocytes were seeded in a decellularized rat liver and cultured for one week in a customized organ chamber with perfusion of the whole-liver through the portal vein. Differentiation and liver-specific function of repopulated cells were examined by comparing the two groups of the livers assembled with the different cell sources.



Results: Immunofluorescence staining at two days showed that fetal hepatocytes differentiated into hepatic marker (albumin and cytochrome P450 subtype [CYP3A4])-expressing cells and biliary marker (cytokeratin 19)-expressing cells with the formation of bile duct-like tubular structures, while there remained α-fetoprotein-positive immature cells. Normalized gene expression levels of albumin and cytochrome P450 subtypes (CYP2E1 and CYP3A11) via quantitative RT-PCR at one week were lower in fetal hepatocyte than those in adult hepatocyte. On the other hand, functional assessment showed significantly higher albumin concentration in the culture medium of the fetal hepatocyte-recellularized liver than the adult hepatocyte-recellularized liver ($p < 0.01$), although urea synthesis was similar.
Conclusions: Fetal hepatocyte engrafted and differentiated into hepatic as well as biliary lineage to construct the three-dimensional architecture in the assembled liver, mimicking the native liver tissue. Furthermore, the fetal hepatocyte-recellularized liver had the non-inferior hepatic function to the adult hepatocyte-recellularized liver, suggesting that fetal hepatocyte is a good candidate for a cell source of liver tissue engineering using a decellularized matrix.

P0011

DIFFERENTIATION OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS INTO HEPATOCYTE-LIKE CELLS AND THEIR REGULATORY EFFECTS ON ACTIVATED LYMPHOCYTES AND LIVER GRAFT REGENERATION AND REJECTION

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Background and Aims: Cell-based tolerogenic therapy is a relatively new approach for the treatment of autoimmune diseases. Bone marrow derived mesenchymal stem cells (BM MSCs) are thought to be an attractive cell source for cell therapy because of their immaturity and low immunogenicity. BM-MSCs are able to differentiate into functional hepatocyte-like cells (HLCs) both *in vitro* and *in vivo*. However, the immunogenic and immunoregulatory effects of differentiated HLCs on lymphocytes and liver grafts *in vivo* have not been reported. To investigate the biological function of HLCs and their effects on activated lymphocytes and liver graft regeneration and rejection.
Methods: HLCs were induced from BM-MSCs and their immunoregulatory effects on activated lymphocytes were investigated. The effects of HLCs transplantation on liver graft regeneration and rejection were investigated in a rat model (BN-Lewis) with 50% reduced-size liver transplantation.
Results: The differentiation of BM-MSCs into HLCs was demonstrated as they exhibited hepatocyte-like morphology and expressed several liver-specific markers. HLCs inhibited lymphocyte proliferation induced by concanavalin A (ConA). Following HLCs transplantation into the rat model with 50% reduced-size liver transplantation, liver function markers and the proportion of hepatocytes in the S-phase of the cell cycle were significantly higher in the experimental group compared to controls ($P < 0.01$). Pathological changes in the HLC-treated groups were less severe than in the control animals.
Conclusions: HLCs inhibited lymphocyte proliferation and exhibited *in vitro* immunosuppressive functions that may be mediated by TGF-β1 and IL-6. HLCs were associated with improved rat liver regeneration and may be beneficial in cell therapies for liver transplant rejection and other liver diseases.

P0012

BILE-LIGATED RATS ARE SUSCEPTIBLE TO HYPOTENSION-INDUCED NEURONAL CELL LOSS: IMPLICATIONS FOR PERSISTING NEUROLOGICAL COMPLICATIONS FOLLOWING LIVER TRANSPLANTATION

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Background and Aims: Hepatic encephalopathy (HE) is a major neuropsychiatric complication caused by liver disease characterized by cognitive and motor dysfunction. The only curative treatment to date remains liver transplantation (LT). Historically, HE has always been considered to be a reversible metabolic disorder and has therefore been expected to completely resolve following LT. However, even following the implantation of a new liver, persisting neurological complications remain a common problem affecting as many as 47% (8–47%) of liver transplant recipients. LT is a major surgical procedure accompanied by intraoperative stress and confounding factors, including blood loss (hypovolumia) and hypotension. We hypothesize, in the setting of MHE, that the compromised brain becomes predisposed to what would normally be an innocuous hypotensive insult, resulting in cell injury and death.
Methods: Six-week bile-duct ligated (BDL) rats with MHE and respective controls will be used. Blood is withdrawn from the femoral artery (inducing hypovolemia) until an arterial pressure of 30 mmHg (hypotension) and maintained for 150 minutes. Upon sacrifice, brains are perfused and extracted for apoptotic analysis (western blot) and neuronal cell count (immunohistochemistry).
Results: Both BDL rats and SHAM-operated controls without hypotension do not display any neuronal loss. However, BDL rats following hypotension demonstrated a significant decrease in neuronal cell count in the frontal cortex using NeuN+DAPI and Cresyl Violet compared to hypotensive SHAM-operated controls. In addition, neuronal loss was associated with an increased in cellular stress protein, hsp32, hsp70 and caspase-3, suggesting apoptotic cell death.
Conclusions: These findings suggest that patients with MHE are more susceptible to hypotension-induced neuronal cell loss. Moreover, these results suggest a patient with HE (even MHE), with a "frail brain", will fare worse during LT and consequently result in poor neurological outcome. The combination of MHE and hypotension may justify for the persisting neurological complications observed in a number of cirrhotic patients following LT. This implies the impact of MHE on outcome is undervalued. MHE should not to be ignored and patients with MHE merit to be treated pre-LT.

P0013

RESOLVIN D1 PROTECTS LIVERS FROM ISCHEMIA/REPERFUSION INJURY BY ENHANCING M2 MACROPHAGE POLARIZATION AND EFFEROCYTOSIS

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Background and Aims: Complete resolution of an acute inflammatory response and its return to homeostasis are essential for tissue integrity and function. Resolution of inflammation is known to be an active process involving a new genus of lipid mediators called specialized pro-resolving lipid mediators such as Resolvin D1 (RvD1). While accumulating evidences suggest RvD1 counteracts proinflammatory signaling and promotes resolution, the specific cellular targets and mechanisms of action for RvD1 remains largely unknown. In the present study, we investigated the roles of RvD1 and its molecular mechanisms in ischemia/reperfusion (IR)-induced sterile inflammation.

Methods: Male C57BL/6 mice were subjected to 70% hepatic ischemia for 60 min followed by reperfusion. RvD1 was administered (5, 10 and 15 μg/kg, i.p.) 1 h prior to ischemia and directly before reperfusion. To deplete Kupffer cells (KCs), mice were pretreated with liposome clodronate (100 μL/mice, i.v.) at 24 h before ischemia. BOC-2, an antagonist for lipoxin A receptor/formyl-peptide receptor (ALX/FPR2), was pretreated (50 μg/kg, i.p.) 30 min before initial RvD1 treatment. The siRNA for ALX/FPR2 (90 μg/kg, i.v.) or control siRNA were pretreated at 72 and 48 h before ischemia.
Results: RvD1 attenuated the IR-induced hepatocellular damage as indicated by serum ALT activity and histopathological score. RvD1 attenuated the increases in the levels of TLR4, TNF-α, IL-6 and IL-1β mRNA expression. In purified KCs of mice exposed to IR, while the levels of M1 marker genes (NOS2a and CD40) increased, M2 marker genes (Arg1, MRC1 and MST1R) decreased demonstrating proinflammatory shift in KCs. RvD1 markedly attenuated these changes. Depletion of KCs by liposome clodronate abrogated the effects of RvD1 on proinflammatory mediators and macrophage polarization. In addition, RvD1 attenuated the increases in the levels of myeloperoxidase activity and CXCL1 and CXCL2 mRNA expression. RvD1 markedly augmented the efferocytic activity (apoptotic neutrophil clearance) of KCs as indicated by increases in $F4/80^{+}Gr\text{-}1^{+}$ cells in liver. However, BOC-2 pretreatment or gene silencing of the RvD1 receptor, ALX/FPR2, abrogated the anti-inflammatory and proresolving action of RvD1.
Conclusions: These results suggest that RvD1 ameliorates IR-induced hepatic injury and this protection is associated with enhancement of M2 polarization and efferocytosis via ALX/FPR2 activation.

P0014
THE EFFECT OF DIFFERENT NUTRITIONAL SUPPORT IN LIVER SURGERY

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Background and Aims: Temporary occlusion of liver blood supply for complex liver operation is common in liver surgery. Steatosis is a risk factor in partial hepatectomy (PH) under ischaemia-reperfusion (I/R), once impairs regenerative response and reduces tolerance to hepatic injury. Nutritional support strategies, as energy source for liver regeneration, remain poorly investigated. We aimed to investigate the effect of different nutritional support on damage and hepatocyte proliferation in liver surgery.
Methods: Male Wistar rats fed with a choline-deficient or standard chow diet for 10 days for nutritional obesity experimental model were used. Steatotic and non-steatotic rat livers were subjected to surgery and the effects of either glucose solution (28%, energy content 4.6 MJ/1000 ml) or lipid emulsion (10%, energy content 4.6 MJ/1000 ml. The emulsion comprised 52% linoleic acid, 22% oleic acid, 13% palmitic acid, 8% linolenic acid, 4% stearic acid, 1% other fatty acids, 8.184 g/l egg phospholipids and 15 g/l glycerine) treatment on damage and regeneration, and part of the underlying mechanisms, were investigated.
Results: In nutritional obesity model, our results revealed an improvement in damage and regenerative process of PH+I/R+28% Glucose and PH+I/R+10% Lipid emulsion in non-steatotic livers when compared with the PH+I/R group and not differences were observed when comparing both treatments. In steatotic livers, glucose (PH+I/R+28% Glucose group) did not protect against damage whereas lipid treatment (PH+I/R+10% Lipid emulsion group) conferred protection. The benefits of these treatments on regenerative failure in steatotic livers were more evident when lipid infusion was administered.
Conclusions: Both nutritional supplements offered the equivalent protection to the non-steatotic liver submitted to PH under I/R. The opposite was seen in fatty liver, since the treatment with lipid emulsion decreased the injurious effects of the hepatic surgical procedure in the presence of steatosis.

P0015
ESTABLISHMENT OF PORTAL VEIN LIGATION + IN SITU SPLITTING MODEL ON RAT

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Background and Aims: Associating Liver Partition with Portal Vein Ligation for Staged Hepatectomy (ALPPS) is an emerging surgical strategy. It induces more and faster liver regeneration of portal non-deprived lobe than that in PVE and PVL, which can induce the rapid growth of future liver remnant (FLR) and reduce post-hepatectomy liver failure (PHLF). However, the underlying mechanism is uncertain. To study the underlying mechanism of liver regeneration and alteration of biochemistry, it is important to establish a reliable ALPPS model on rats.
Methods: Portal vein ligation was achieved by occlusion of portal branches feeding left liver lobe and right lobe (approximate 70%PVL). In situ splitting was performed according to the portal fissure. A sterile plastic film was placed on the transection surface of left median lobe. The caudate lobes were resected (10%PHx), simulating the clinical situation of atypical resection during first stage. The liver regeneration of right median lobe was evaluated. The model was tested by imaging techniques (CT scan and 3D reconstruction) after harvest on day 7.
Results: Rats can survive 100% after performing the procedure correctly. The future remnant liver (right median lobe) grew significantly (200% of initial size). 3D reconstruction showed the right median portal vein was well reserved, while the rest portal veins were properly ligated. All hepatic veins were well reserved. No obvious revascularization was found between left and right median lobe.
Conclusions: Based on the anatomy of rodent liver, the ALPPS model can be performed reliably and well-tolerated. The whole procedures included the ligation of left portal vein, transection of median lobe along the portal fissure and resection of caudate lobe.

P0016
VISUALIZATION OF LIVER REGENERATION AFTER 70% PARTIAL HEPATECTOMY IN MICE

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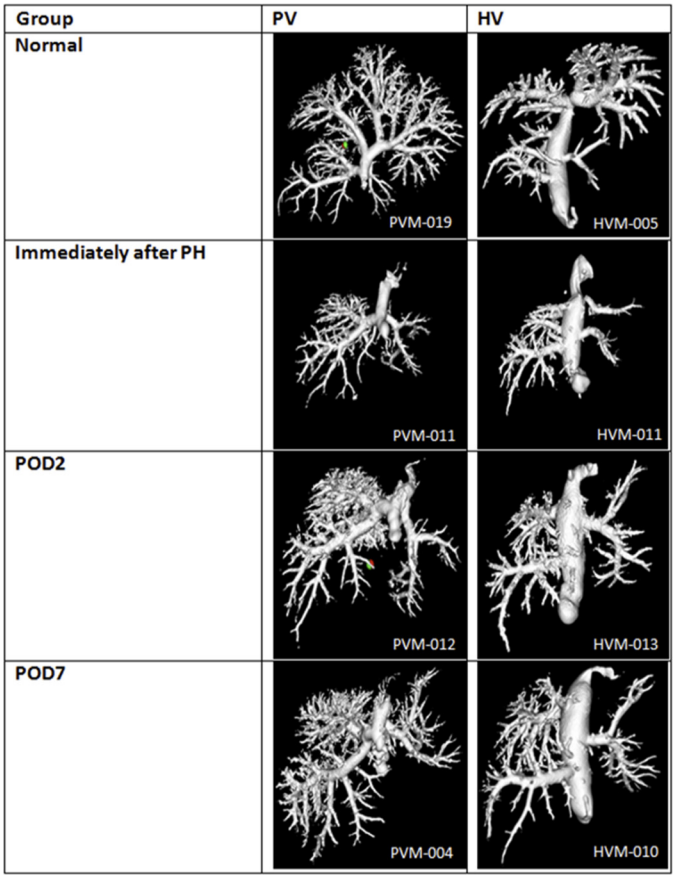
Background and Aims: Mouse partial hepatectomy model is the most common used model to investigate liver regeneration. However, little is known about the kinetics of vascular regeneration after liver resection in mice. The aim of this study was to
1. Establish a method to visualize vascular regeneration by injecting a contrast agent (Microfil) and subsequent CT-imaging of the explanted regenerating mouse liver.

2. Observe kinetics of vascular regeneration of portal and hepatic vein after 70% PH in mice.

Methods: *Specimen preparation:* Laparotomy was performed on anesthetized mice before, immediately after and on POD2 and POD7 after 70%PH (n = 6/time point). After systemic heparinization, portal veinwas cannulated with 26G heparinized catheter and flushed with heparinized saline to remove the blood in liver and to prevent blood clotting using a speed-controlled perfusion device (0.4 ml/min). The mice were euthanized thereafter. Next, two specimen preparation methods were utilized: (1) portal vein vascular system: microfil reagent was perfused into portal vein after heparin perfusion via existing catheter (0.2 ml/min). (2) Hepatic venous system: infrahepatic inferior vena cava (IVC) was cannulated with another 26G catheter. Microfil component was perfused into IVC to hepatic vein through this catheter after the branches of IVC were ligated and suprahepatic inferior vena cava was clamped.
Micro-CT scanning: The microfil samples were subjected to CT scanning using SkyScan 1172 (Tomoscope Duo, CT-Imaging, Erlangen, Germany) μCT system (SkyScan, Kontich, Belgium) as described by Josef Ehling.

Results: *Micro-CT images:* Using the method described above, we could obtain micro-CT images suitable for 3D-reconstruction. Comparing the Micro-CT images obtained on POD2 with those obtained immediately after PH group, the vessel diameter doubled by POD2. In contrast, the number of third order branches from both portal and hepatic venous tree did increase by POD2, but even more by POD7, leading to an increased vascular density.

Conclusions: CT imaging of explanted livers after microfill contrasting the vascular tree represents a useful tool for visualizing vascular regeneration after partial hepatectomy. Vascular growth consists of enlarging the diameter of the vascular stem with its main branches and outgrowth of additional terminal branches in both the portal venous and hepatic venous tree.



P0017

DEVELOPMENT OF TISSUE-ENGINEERED VASCULARIZED PORCINE LIVER SCAFFOLDS FOR HUMAN TRANSPLANTATION

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Background and Aims: There is an ever-rising demand for transplantable livers in end-stage liver failure patients for replacement of damaged livers. The field of regenerative medicine could provide a solution through engineering of bioartificial livers. Our aims are to establish an optimized protocol for constructing a bioartificial hepatic lobe, investigate its immunocompatibility and create a humanized recellularized hepatic scaffold using porcine livers.

Methods: Decellularization of the right lateral lobe was performed by perfusing sodium dodecyl sulfate through the right branch of portal vein. Decellularized lobes were evaluated by morphologic and biochemical techniques. DNA content was quantified to validate the decellularization protocol. Presence of α 1,3-galactosyltransferase, major histocompatibility complex, porcine vonWillebrand Factor and sialoadhesin was checked to exclude the potential rejection and thrombosis after transplantation. Additionally, porcine endogenous retrovirus, a well-known zoonotic risk, was analyzed. Xeno-reactivity of decellularized tissue against human PBMCs was also examined. We recellularized this lobe by HepG2 cells and human endothelial cells via portal vein, then cultured inside a bioreactor for 10 days. Perfusion of recellularized scaffolds with human blood was performed to check the patency of vessels. Distribution of seeded cells within scaffolds was analysed by histology. Immunohistochemistry and PCR were performed for evaluating the functions and proliferating abilities of cells grown within scaffolds.

Results: A translucent lobe with an intact vascular network was successfully developed. H&E staining showed complete removal of the cellular materials and preserving the intact architecture. Although resulting matrices maintained the complex composition of ECM, approximately 99% of DNA was eliminated. The scaffolds were depleted from immunogenic and viral antigens. Human immune response against scaffolds was non-significant. After recellularization, HepG2 cells were almost evenly distributed within scaffolds. No thrombosis was observed after human blood perfusion, indicating a fully endothelialized vascular tree. Immunohistochemistry and PCR confirmed that seeded cells retain their functions and proliferating abilities.

Conclusions: Decellularized porcine liver provides a tissue engineering platform that can be used potentially after recellularization and endothelization as a liver support system or direct transplantation in human.

P0018

ADENOSINE PROTECTS LIVER AGAINST OXIDATIVE STRESS DAMAGE IN LIVER ISCHEMIA/REPERFUSION (I/R) INJURY

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Background and Aims: Ischemia/reperfusion (I/R) injury is a multifarious process detrimental to liver graft function. Mechanisms involved in I/R injury are essential for the design of therapeutic strategies to improve the outcome of liver transplantation. The inflicts tissue damage and initiates. There are many reports on generation of reactive oxygen species

subsequent to re-oxygenation and they initiate a cellular cascade leading to inflammation, cell death, and possible organ failure. Complex manifestations such as rapid depletion in cellular ATP, increase in cytosolic calcium, activation of phospholipases and proteases, infiltration of neutrophils as well as arachidonic acid metabolism may reflect changes in the steady-state concentration of pro/antioxidants resulting in oxidative stress. The aim of this study was to examine the effect of adenosine preconditioning in protecting against a hepatic I/R injury

Methods: We have studied the lipid peroxidation levels (as MDA), glutathione (GSH) levels, catalase (CAT) and glutathione peroxidase (GPx) activity by appropriate spectrophotometric methods, in liver homogenate of male rabbits, one month old, who were allocated into two groups. The first group I/R animals were subjected to 45 min of right-lobe hepatic ischemia, followed by 48 hours of reperfusion. The second group intraportally received infusion of adenosine in physiological solution in dose of 1 mg/kg 20 min before I/R damage. The animals were sacrificed 48 h afterwards, livers were quickly removed, frozen and homogenised on ice.

Results: Obtained results demonstrated that MDA level was significantly decreased in second group compared to I/R group ($p < 0.001$). The levels of GSH as well as CAT activities were increased in adenosine treated group ($p < 0.001$ vs. I/R group). The results also showed statistically significant decrease activity of GPx ($p < 0.001$ vs. I/R group).

Conclusions: These results suggest that intraportally infused adenosine attenuates reperfusion injury of the liver, presumably by suppressing the activation of neutrophils and oxidative stress, so adenosine preconditioning can have protective effect during liver transplantation.

P0019

ADIPONECTIN AND RELATED ADIPOCYTOKINES IN STEATOTIC AND NON-STEATOTIC LIVER TRANSPLANTATION

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Background and Aims: Controversial roles for adiponectin and related adipocytokines have been described in different liver pathologies, nevertheless it is unknown their possible implication in ischemia-reperfusion injury associated with liver transplantation. Our study aimed at characterizing the role of the adiponectin-derived molecular pathway in transplantation with steatotic and non-steatotic liver grafts

Methods: Steatotic and non-steatotic liver transplantationwas carried out and the hepatic levels of adiponectin, visfatin and resistin were measured and modulated either pharmacologically

Results: Pharmacological strategies aimed at modulating adiponectin or resistin were irrelevant for non-steatotic liver grafts submitted to 6 h of cold ischemia. However steatotic livers are more predisposed to downregulate both adiponectin and resistin when subjected to transplantation. Visfatin is not involved in either the vulnerability of steatotic and non-steatotic livers to damage associated with transplantation or in the benefits of adiponectin in steatotic liver transplantation. Adiponectin pre-treatment only protected steatotic grafts and did it so through a resistin-dependent mechanism. Adiponectin-derived resistin accumulation activated the PI3K/Akt pathway. Strategies aimed at increasing adiponectin prevented the downregulation of PI3K/Akt pathway and protected steatotic liver grafts. Conversely, PI3K/Akt pathway upregulation and hepatic protection induced by adiponectin were abolished when resistin action was inhibited

Conclusions: Our findings reveal a protective pathway in steatotic liver transplantation, which may help develop new strategies aimed at increasing either adiponectin or resistin in the steatotic liver undergoing transplant to ultimately increase organ donor-pool for transplantation since they may improve the outcomes of steatotic liver grafts that otherwise would not be transplanted

P0020

ROLE OF ENDOTHELIAL AUTOPHAGY IN LIVER ISCHEMIA AND REPERFUSION INJURY

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Background and Aims: The liver endothelium is highly susceptible to Ischemia/Reperfusion (I/R) injury, thus its preservation determines the success of transplantation. It is unknown wether Autophagy, an intracellular process that controls cellular homeostasis and survival, plays a role in liver endothelial I/R injury. Simvastatin, a vasoprotective compound that preserves liver microcirculation through a KLF2-dependent and cholesterol-independent manner, has recently been shown to activate autophagy.

Aims: (1) Characterize autophagy in liver sinusoidal endothelial cells (LSEC) cultured under I/R conditions, and its effects on cell viability, and (2) evaluate the role of autophagy in the protection of the liver microcirculation conferred by simvastatin, and its underlying molecular pathways, using *in vitro* and *ex vivo* models of hepatic I/R injury.

Methods: *in vitro*: primary LSEC where pretreated with simvastatin (KLF2 inducer; 5μM), GGPP (KLF2 inhibitor; 1μM) and/or chloroquine (autophagy inhibitor; 20μM) and cultured mimicking I/R [6 h, 12 h or 24 h in University of Wisconsin solution (UWS) or Celsior at 4°C + 2 h of warm reperfusion; n = 3 per condition]. Determinations: Autophagic flux as LC3 levels in presence or absence of chloroquine, cell death by acridine orange and propidium iodide staining, KLF2 levels by western blot.

Ex vivo: Wistar rats were treated with simvastatin (1 mg/kg i.v.), chloroquine (60 mg/kg i.p.) + simvastatin, or vehicle (n = 8 per group). 30min later livers were explanted, cold stored in UWS for 16 h and, and after 2 h of reperfusion hepatic microvascular function was characterized.

Results: *In vitro*: LSEC cold stored in Celsior solution exhibited a time-dependent activation of autophagic flux. However, no changes in autophagic flux were observed in UWS-stored LSEC due to an inhibited formation of autolysosomes. Simvastatin re-activated autophagy in UWS-stored cells, which in turn increased KLF2 expression, and ultimately improved cell viability. The beneficial effects of simvastatin were blunted when autophagy or KLF2 expression were previously inhibited.

Ex vivo: simvastatin ameliorated the microvascular function of UWS-stored liver grafts. This effect was markedly diminished when hepatic autophagy was inhibited.

Conclusions: The present study describes for the first time two concepts: (1) activation of endothelial autophagy as a protective strategy during organ cold storage for transplantation, and (2) induction of the transcription factor KLF2 through the activation of autophagy.

P0021

ALTERING TRANSPLANTED CELL ENGRAFTMENT AND PROLIFERATION IN THE LIVER THROUGH PARACRINE SIGNALING WITH COTRANSPLANTATION OF LIVER SINUSOIDAL ENDOTHELIAL CELLS AND HEPATOCYTES IN MICE

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Background and Aims: Liver is maintained in a healthy state by tightly regulated interactions among liver cell types. For instance, liver sinusoidal endothelial cells (LSECs) are able to initiate and sustain liver regeneration process. After cell transplantation, multiple cell compartments are activated and cell-cell interactions regulate transplanted cell engraftment and proliferation. Activation or disruption of LSEC facilitates engraftment of transplanted hepatocytes (HEP), as well as LSEC. To develop strategies for liver-directed cell and gene therapy, insights into such cell-cell interactions will be helpful, particularly for improving transplanted cell engraftment, survival and proliferation. Recently, we demonstrated that under suitable conditions transplanted LSEC can engraft, function and reconstitute the hepatic endothelium. We studied potential roles of LSEC in engraftment and liver repopulation of transplanted HEP.
Methods: We used C57Bl/6, GFP^+ and $DPPIV^-$ mice as HEP and LSEC donors and $DPPIV^-$ mice as recipients treated with monocrotaline alone or in combination with riphampicin and phenytoin, which improves transplanted cell engraftment and proliferation. In some experiments LSEC were transduced withlentiviral vectors expressing VEGF or HGF. We compared the gene expression profile of genes specific for ECs biology in the transplanted livermice.
Results: After 1w cotransplantation improved the overall cell engraftment. After 6w the liver endothelium of recipient mice was replaced by transplanted LSEC and the overall HEP proliferation was not improved compared to mice transplanted with HEP alone. After LSEC genetic manipulation, in vitro experiments showed that LSEC-released factors promoted HEP proliferation or viability, while in vivo improvedHEP engraftment, demonstrating thatnative LSEC were not providing enough paracrine factors and so additional manipulations are needed to improve HEP engraftment. Gene expression profile analysis of EC specific genes 6, 24, 48 h and 1w post-transplant showed that LSEC transplantation does not perturb the liver microenvironment at any time point, while changes were detected after HEP infusion or cotransplantation, meaning that major changes can be attributed to HEP.
Conclusions: The ability to reconstitute the liver with LSEC and HEPco-transplantationwill offer potent ways to investigate biological mechanisms of cell-cell interactions to develop protocols for cell therapy of suitable disorders.

P0022

WHOLE ORGAN ENGINEERING USING DECELLULARIZED SCAFFOLD FOR LIVER TRANSPLANTATION IN LARGE ANIMAL MODEL

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Background and Aims: Fulminant hepatic failure remains a serious clinical condition that is associated with a high mortality rate. The only effective treatment is liver transplantation, however, there is currently a severe shortage of donor livers. Therefore a novel methodology to replace organ transplant and shift to regenerative approaches is strongly required. The organ decellularization can produce the native organ scaffold which can carrier any type of cell source include iPS derived cells. These scaffolds are generated by removing viable cells from organ and the vascular structure was preserved, which can be connected to native vascular system by surgical anastomoses. It is important to find the feasibility of this technology to be applied into human scale and to see biological alterations of the scaffold after implantation.
Methods: We demonstrated that the decellularization methodology could be applied in large animal model and it could be sufficiently recellularized with endothelial cells and hepatocytes. The decellularized scaffold was generated by procedure using Trypsin, SDS, TrytonX-100 and CHAPS. To prevent coagulation after blood perfusion, endothelial cells and hepatocytes were infused into the scaffold with monitoring intravascular pressure and perfusion flow rate. In addition we also infused the amphipathic polymer having an anti-coagulant activity into the scaffold. Blood flow was evaluated by angiography after vascular anastomoses after transplantation. Histological study was performed after transplantation to evaluate cell infiltration, degree of coagulation and adhesion around the scaffold.
Results: The endothelial cells could cover most of the vessel lumen of the scaffold and the hepatocytes were well distributed into the parenchymal space. We could successfully transplant the recellularized graft in porcine body by vessel anastomosis of portal vein and IVC without any leakage. Although histological study showed that the coagulation was not completely avoided of the vascular system in the graft, the coagulation was less in the area sufficiently covered with the endothelial cells and the hepatocytes. The graft was well perfused and preserved in the porcine abdominal cavity without bleeding or absorption.
Conclusions: Although it requires improvement and customization in point of anti-coagulation, we could scale-up and optimize the system to apply this unique technology for clinical applications.

P0023

COMPARISON OF THE C3A AND HepaRG LIVER CELL LINES IN MONOLAYER AND 3D FOR BIOARTIFICIAL LIVER APPLICATION

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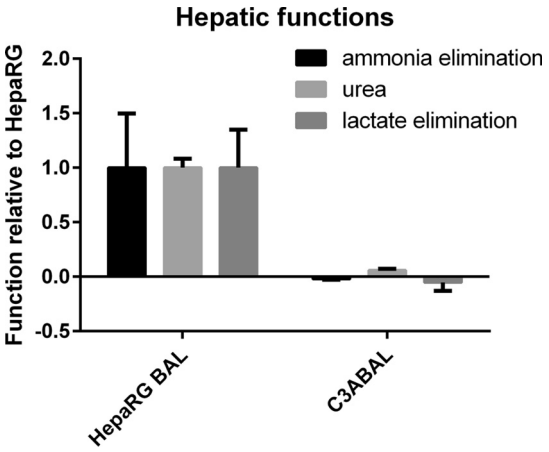
Background and Aims: Recently, the first clinical trials of Bioartificial livers (BAL) loaded with a proliferative hepatocyte source have started. C3A cells are among the most commonly deployed cell lines in BAL devices. However, we and others have suggested that the hepatic progenitor cell line HepaRG exhibits a phenotype that reflects the phenotype of primary hepatocytes to a significantly higher extent compared to C3A cells, which would render this cell line more suitable for BAL application. No head-to head comparison between C3A and HepaRG is available yet. Therefore, we directly compared parameters relevant for BAL therapy of both cell lines, in monolayers and laboratory sized BAL devices.
Methods: First we defined the optimal culture and differentiation conditions for C3A cells (acquired from ATCC) in monolayers and in laboratory sized AMC miniBALs. Next, we compared the C3A cells with HepaRG cells both differentiated in optimal conditions in the same culture medium in monolayer and miniBALs.
For comparison, the cells were assessed at biochemical level for ammonia elimination, urea cycle activity, albumin production, AST and LDH leakage, amino acid metabolism, glucose and lactate metabolism. The results were normalized to total protein content. In addition the cells were assessed for transcript levels of hepatic genes.

Results: Both cell lines reached a higher differentiation grade in BALs compared to monolayer. HepaRG cells outperformed C3A cells for most tested parameters. Most notably, C3A cells produced lactate and ammonia whereas HepaRG consumed both compounds when cultured in a BAL. In addition C3A cells consumed glutamine while HepaRG cells showed pronounced production, suggesting high activity of glutaminase in C3A cells and more active glutamine synthase in HepaRG cells. Gene expression levels for xenobiotic metabolism and membrane transporters were 15–200 fold higher in HepaRG. Expression of the fetal marker AFP was up to 10^4 fold higher in C3A cells. Cell leakage was significantly higher in C3A and albumin synthesis was comparable between the cell lines.
Conclusions: Both cell lines perform better in the environment of a BAL as compared to monolayers. HepaRG cells significantly outperform C3A cells on a multitude of parameters relevant for BAL application. These results thus suggest that HepaRG may be the more suitable biocomponent for BAL application.



P0024
EFFICACY OF WHARTON'S JELLY-DERIVED MESENCHYMAL STEM CELLS COMBINED WITH PRAZIQUANTEL IN SCHISTOSOMA MANSONI-INDUCED LIVER FIBROSIS IN MICE
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Background and Aims: Several studies have demonstrated the role of mesenchymal stem cells in the hepatic regenerative process. The aim of the study was to investigate the feasibility of liver damage repair using Wharton's jelly-derived mesenchymal stem cells (WJMSCs; the major umbilical cord stem cell population) combined with praziquantel (PZQ) to treat Schistosoma (S.) mansoni-induced liver fibrosis.
Methods: Mice received early (8th week post infection) and late (16th week post infection) intra-hepatic injection of WJMSCs, alone or combined with oral PZQ, to investigate the treatment efficacy on both acute and chronic stages of liver fibrosis, respectively. Histopathological, morphometric, and immunohistochemical analysis for alpha fetoprotein, alpha smooth muscle actin, Hep par-1, cytokeratin-18, vimentin, and β2-globulin, were performed. Relative mRNA expression of albumin, alpha fetoprotein, alpha smooth muscle actin, collagen I, and interleukin 13 was measured by real time reverse transcription polymerase chain reaction (RT-PCR). Gelatin zymographic analysis for matrix metalloproteinase (MMP)-2 and 9 was also performed. The previously mentioned studies were used to monitor transplanted stem cell differentiation into hepatocyte-like cells and to assess S. mansoni-induced liver fibrosis.
Results: Histopathological and morphometric findings showed a regression in fibrosis in WJMSCs-treated groups and better results were obtained when PZQ was combined to stem cell therapy. Immunohistochemical and RT-PCR findings showed positive expression for hepatocyte-specific markers in transplanted groups and an amelioration of fibrosis-related markers. Gelatin zymography results showed an elevation of the enzymatic activity of MMP-2 and -9 in WJMSCs-treated groups. Meanwhile, PZQ caused a reduction in the activity of both enzymes. Combined treatment, however, caused no or little change
Conclusions: The differentiation of transplanted WJMSCs into functioning hepatocyte-like cells in the livers of S. mansoni-infected mice may have contributed to partial repair of liver fibrosis, especially when PZQ is administered concomitantly

Liver transplantation/Surgery: b. Clinical

P0025
THE PUBLIC HEALTH VALUE OF SPARING LIVERS FOR TRANSPLANTATION THROUGH SYSTEMATIC TREATMENT OF HEPATITIS C
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Background and Aims: The increasing availability of highly efficacious new treatments for hepatitis C virus (HCV) is expected to significantly reduce the number of people progressing to end stage liver disease (ESLD) and becoming candidates for liver transplantation in the future. Our study aims to quantify the potential benefits for ESLD patients waiting for liver transplants (LTs) in the US beyond those who have HCV. Several studies have modeled the potential changes in screening and treatment pathways likely to result from new HCV treatment introductions, but these models have been developed primarily to assess health outcomes in HCV patients such as decompensated cirrhosis, hepatocellular carcinoma, and liver related death. Moreover, the effect of new treatments on the availability of future transplants depends on the complex interaction of HCV epidemiology, the prevalence of other diseases – most notably alcoholic liver disease, and nonalcoholic fatty liver disease (NAFLD) – and on the future supply of healthy livers for transplantation.
Methods: To ensure robustness, we used multiple methods to estimate future demand for livers between 2015 and 2035. We used a Markov model to study a scenario of widespread screening and treatment for HCV implemented from 2015 to 2020. The Markov model results were validated against those of an attributable risk model based on both Organ Procurement and Transplantation Network and National Health and Nutrition Examination Survey data. We also considered a number of scenarios for future disease trends, ranging from stable prevalence of non HCV liver disease

to projected growth in prevalence based on empirical evidence of trends in risk factors such as obesity and diabetes, the primary drivers of NAFLD incidence.
Results: The projected cumulative number of LTs averted for ESLD patients due to highly effective HCV treatment is estimated to be 10,490 over the period 2015–2035 (see Figure). Of this total, 7,320 livers would accrue to patients without HCV, accounting for 9% of livers used for all non-HCV liver transplants over this time period and approximately 123,700 life-years gained by non-HCV patients.
Conclusions: More systematic use of highly effective treatments for HCV in the US may have substantial public health benefits for patients suffering from ESLD due to decreased liver transplantation in the HCV population. Most of these benefits accrue to patients without HCV.

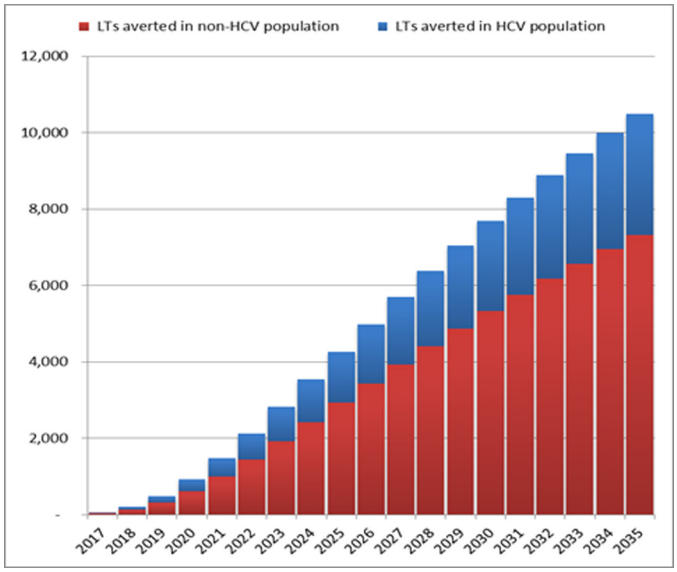

Figure: Cumulative estimation of liver transplants (LTs) averted from years 2015–2035 in the US from a scenario of introducing widespread screening and treatment of HCV in 2015–2020.

P0026
THE HIV INFECTION DOES NOT HAVE IMPACT ON SURVIVAL AND TUMOR RECURRENCE IN HEPATOCELLULAR CARCINOMA PATIENTS TREATED BY LIVER TRANSPLANTATION

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Background and Aims: Previous studies including a limited number of patients and/or short follow-up offer conflicting data regarding the impact of HIV on survival in patients with hepatocellular carcinoma (HCC) after liver transplantation (LT). The aim of this prospective multicenter study was to evaluate the influence of HIV infection in tumor recurrence and post-LT survival in HCC patients treated by LT.
Methods: Prospective cohort study including HIV infected patients undergoing LT for HCC in 22 Spanish hospitals (cohort FIPSE) and transplanted HIV (−) patients matched for age, gender, year of LT, HCV, HBV and HCC in a ratio 1: 3. Incidental HCC patients were excluded. Enrollment began in January 2002 and the end of follow-up was in July 2014.
Results: 74 patients HIV (+) patients and 222 HIV (−) underwent LT for HCC during the study period. Most were men (86%) and had HCV co-infection (92%). HIV (+) patients were younger (47 vs. 51 years, $p < 0.001$) and had genotype non-1 with higher frequency (55 vs. 25%) and less frequent HCV replication at the time of LT (80% vs 90%) compared to HIV (−) patients. At the time of LT, median [interquartile range] CD4/mm3 were 347 [238–523] and the majority of HIV (+) patients (96%) were on antiretroviral therapy. The HIV viral load was <50 copies/ml in 93%. There were no differences in waiting time until the LT (4.5 vs. 5.4 months, $p = 0.35$), in baseline HCC characteristics at the moment of waiting list inclusion and on explant histological parameters between both groups. After a median follow-up of 46 [25–72] months, 12 (16%) HIV (+) patients and 32 (14%) HIV (−) patients had tumor recurrence. The probability of recurrence at 1, 3, and 5 years in HIV (+) and HIV (−) was 7%, 17% and 20% vs. 5%, 11% and 19%, respectively ($p = 0.876$). Analyzing jointly HIV patients (+) and HIV (−), microscopic vascular invasion (HR, 3.79; 95% CI, 1.67 to 8.57) was the only factor independently associated with HCC recurrence. Survival at 1, 3 and 5 years for HIV (+) patients compared to HIV patients (−) was 87%, 78% and 69% vs. 89%, 78% and 73% ($p = 0.905$). HCV infection (HR, 8.85 95% CI, 1.23 to 63.64) and the presence of satellite nodules (HR, 1.92; 95% CI, 1.13 to 3.24) were the only variables independently associated with mortality.
Conclusions: HIV infection has no impact on HCC recurrence or survival after LT. These results support the indication of LT in HIV-infected patients with HCC.

P0027
COST-EFFECTIVENESS OF PRE-TRANSPLANT SOFOSBUVIR TO PREVENT RECURRENCE OF HCV INFECTION AFTER LIVER TRANSPLANTATION

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Background and Aims: The strong efficacy of pre-transplant Sofosbuvir (SOF) plus Ribavirin in preventing recurrence of hepatitis C virus (HCV) infection after liver transplantation (LT) has been reported. The aim of this study was to evaluate the cost-effectiveness of this strategy in the North Italy Transplant program (NITp) area.
Methods: We first evaluated the impact of HCV infection on post-LT survival in 2376 consecutive adult patients (MELD ≤25, period 2004–2009, NITp area) and prevalence-costs of conventional standard of care (SOC) antiviral therapy (Peginterferon plus ribavirin) after LT. A Markov model was developed to compared two strategies: SOF + Ribavirin (RBV) as pre-LT anti-HCV treatment (strategy A), versus on-demand post-LT SOC antiviral therapy (strategy B). The endpoint was incremental cost-effectiveness ratio (ICER) as costs ($)/quality-adjusted life year (QALY).
Results: Among the 1794 patients undergoing LT and meeting the inclusion criteria, 860 (48%) were HCV+ and 50% of them received SOC therapy post-LT (drugs and adverse effects management mean costs = 16,440$ for patient). HCV etiology had a strong impact on post-LT survival (hazard ratio 1.59, 95% CI 1.22–2.09, $p = 0.0007$). After Monte Carlo simulation, pre-LT SOF therapy showed a median

survival benefit of 2.0 QALYs, and an ICER of 33,600$/QALY. Costs of SOF therapy, sustained viral response rate 12 weeks after LT, and recipient age were the main ICER predictors at multivariate analysis.

Conclusions: Pre-transplant SOF in HCV patients proved to be a cost-effective treatment compared to post-LT SOC therapy.

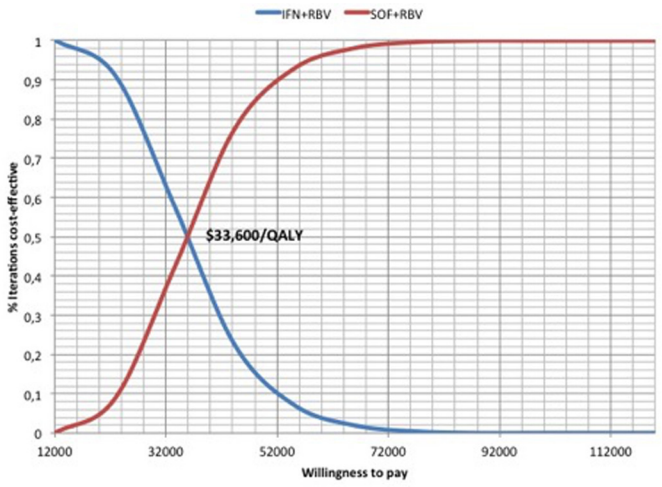

Figure: Cost effectiveness acceptability curve.

P0029

ACTIVE IMMUNIZATION IN PREVENTION OF DE NOVO HEPATITIS B IN ADULT LIVING DONOR LIVER TRANSPLANTATION WITH CORE ANTIBODY POSITIVE GRAFT

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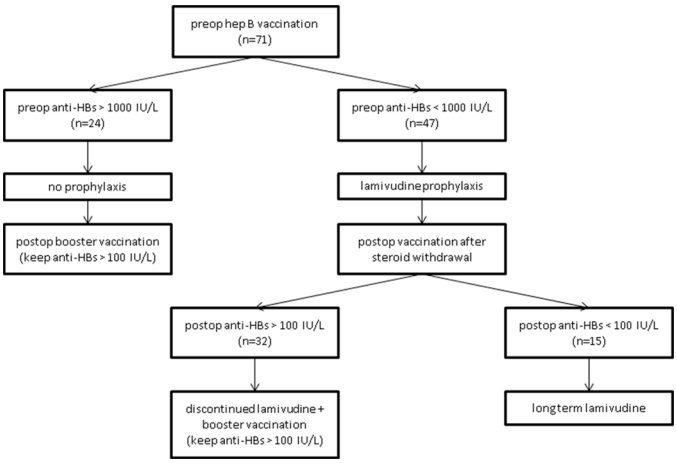
Background and Aims: De novo hepatitis B infection (DNHB) can occur in transplant recipients who receive organs from anti-HBc(+) donors. Post transplant prophylaxis can reduce the risk of DNHB. However, the issues of development of antiviral drug resistance and high cost of HBIG make it to be impractical for long term use. Studies on active immunization in paediatric population have shown promising results. However, its effect in adults is still uncertain. Our aim is to investigate the use of HBV vaccination as an alternative to prevent DNHB in adult living donor living transplantation.

Methods: Patients (with age ≥18 years) who received anti-HBc(+) liver grafts in a single center were enrolled. The cohort was stratified into 3 groups according to the response to HBV vaccination (flow chart). Group 1: responder to pre transplant vaccination with anti-HBs titer >1000 IU/L at the time of transplantation and for post transplant surveillance only; Group 2: no response to pre transplant vaccinations but responded to post transplant vaccination with anti-HBs titer >100 IU/L after transplantation, lamivudine was stopped after a minimum period of 2 years; Group 3: no response to pre and post transplant vaccination, lamivudine was continued indefinitely.

Results: A total of 71 patients were enrolled (group 1: 24, group 2: 32 and group 3: 15). All recipients with high pre transplant anti-HBs titers (group 1) achieved post transplant anti-HBs level >100 IU/L. Groups 1 and 2 had significant higher DNHB-free survival rates than group 3 (gp 1 vs 3: $p = 0.016$, and gp 2 vs 3: $p = 0.004$ respectively). All 3 cases of DNHB were from group 3, none was detected in groups 1 and 2 after a median follow-up of 61 months.
Higher recipient and donor pre transplant anti-HBs titer, post transplant achievement of anti-HBs >100 IU/L, and higher post transplant peak anti-HBs titer were associated with reduced risk of DNHB on univariate analysis ($p < 0.05$). However, multivariate analysis using cox regression analysis failed to find significant association.

Conclusions: Active immunization is effective in preventing DNHB in adult living donor liver transplantation, by keeping pre transplant anti-HBs level of >1000 IU/L and post transplant anti-HBs of >100 IU/L. With vaccination, lamivudine can be stopped in patients who achieve post transplant anti-HBs level of >100 IU/L. Hence, active immunization provides a cheaper and safe alternative to existing regimens.



P0030

UTILITY BASED CRITERIA TO SELECT PATIENTS WITH HEPATOCELLULAR CARCINOMA FOR LIVER TRANSPLANTATION: A MULTICENTRE COHORT STUDY

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Background and Aims: The lifetime utility of liver transplantation (LT) in patients with hepatocellular carcinoma is still controversial. The aim of this study was to understand when LT is cost-effective for HCC patients in order to suggest new potential transplant selection criteria.

Methods: The overall design of this study involved a real cohort of transplantable Italian HCC patients (n = 2419 selected from the ITA.LI.CA database) undergoing non-transplant therapies for whom a survival analysis and calculation of direct costs of therapies were performed, and then a Markov model to calculate the survival benefit and cost-utility of LT over non-LT therapies. Post LT survival was calculated using the α-fetoprotein (AFP) model based on AFP values and radiological size and number of nodules. Primary endpoint was Net Health Benefit (NHB), defined as LT survival benefit in quality adjusted life years (QALYs) minus incremental costs (#)/willingness to pay ($32,946, corresponding to the Italian Gross Domestic Product).

Results: We developed a prognostically accurate survival model predicting non-LT survival, based on AFP model variables, Child Pugh classes, and type of alternative therapy (resection, loco-regional, or best supportive care). The calculated median cost of non-LT therapies per patient was $21,670. The Monte Carlo

simulation showed that in patients with resectable child A HCC, the NHB of LT was always negative, independently from the AFP model values, while in unresectable HCC patients, the NHB of LT was positive for AFP model values ≤4, and negative for values >4.
Conclusions: LT proved to be cost-effective in HCC patients with unresectable tumor and AFP model values ≤4.

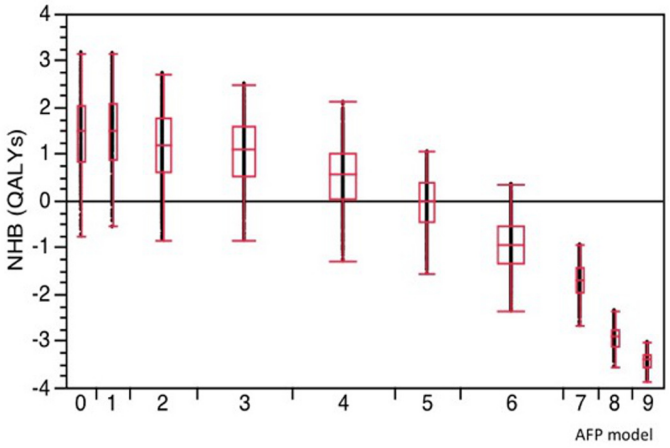

Figure: NHB of liver transplantation in unresectable HCC patients.

P0031
FREQUENCY AND OUTCOMES OF SIMULTANEOUS LIVER KIDNEY TRANSPLANTATION IN NASH

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Background and Aims: Frequency of liver transplants (LT) is increasing in non-alcoholic steatohepatitis (NASH) with better outcomes compared to other etiologies. Similar data on simultaneous liver kidney (SLK) transplants are limited.
Methods: UNOS database (2002–2011) queried for deceased donor first LT for primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hepatitis C virus (HCV), alcoholic liver disease (ALD), combined ALD and HCV, NASH, cryptogenic cirrhosis (CC), hepatitis B virus (HBV), and hepatocellular carcinoma (HCC) and stratified to LT alone (LTA) and SLK. LT indication was stratified to PBC+PSC+ALD (group I), NASH+CC (group II), and HCV±ALD+HBV+HCC (group III).
Results: Of 40,326 analyzed LT (9495, 5448, and 25,383 in groups I–III respectively), about 5.8% (N = 2319) received SLK (6%, 9%, and 5% in groups I–III respectively). Of LTA, 22%, 14%, and 64% were performed for group I–III respectively. Similar respective figures of all SLK were 23%, 20%, and 57% respectively (Figure). Frequency of SLK increased from about 4% in 2002 to about 7% in 2011. Trends for group I–III were 4% to 5.8%, 4% to 10%, and 4% to 8% respectively. SLK for NASH+CC (group II, n = 477) compared to 1842 non-NASH SLK (group II+III) were older, more likely to be females, diabetics, and Caucasians, and have higher body mass index. Five year respective outcomes after SLK comparing group I–III were 78% vs. 76% vs. 66% for liver graft, 79% vs. 72% vs. 65% for kidney graft, and 81% vs. 77% vs. 69% for patient survival, Log Rank P < 0.0001 for all. Comparing groups I and II, outcomes were similar for liver graft (P = 0.29) and patient survival (p = 0.14) but worse for group II on kidney graft (P = 0.01). Diabetics compared to non-diabetics had worse five year kidney survival for non-NASH SLK (67% vs. 71%, P = 0.044). Similar respective figures among group II were 69% vs. 78%, P = 0.017. Patients receiving SLK for NASH or CC (group II) were 29% more likely to lose kidney graft [1.29 (1.002–1.67)] compared to non-NASH transplants after controlling for recipient characteristics and kidney donor risk index. Other strong predictors were black race [1.32 (1.06–1.63)] and dialysis [1.26 (1.07–1.49)].
Conclusions: Frequency of SLK transplants is increasing among NASH patients requiring liver transplantation. SLK recipients for NASH have worse renal outcomes independent of associated diabetes. Studies are needed to examine mechanisms of renal pathology in NASH and to develop strategies to improve renal outcomes in NASH patients receiving SLK.

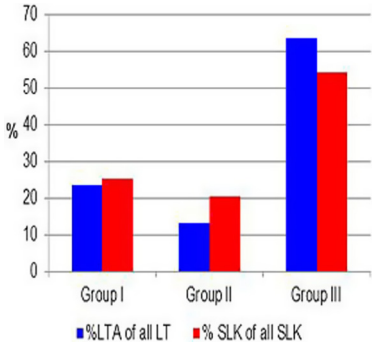

Figure: LTA (blue bars) and SLK (red bars) for specific etiology as proportion of all LTA and SLK respectively.

P0032
THE IMPACT OF ISCHEMIA/REPERFUSION INJURY ON LIVER ALLOGRAFTS FROM DECEASED AFTER CARDIAC DEATH VERSUS DECEASED AFTER BRAIN DEATH DONORS

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Background and Aims: The continuing shortage of appropriate organs for transplantation has led to the reevaluation and use of organs procured from donors after cardiac death. The pathophysiologic processes associated with cardiac as compared to brain death are poorly understood. The aim of this study was to determine how the ischemia/reperfusion (I/R) event associated with transplantation affects allografts obtained from donors after cardiac death (DCD) versus donors after brain death (DBD).
Methods: Allograft leukocyte infiltration and expression of inflammatory and cell death markers were compared between DCD (n = 22) and DBD (n = 13) livers by immunohistochemical (IHC) analysis using pre- and post-reperfusion liver biopsies. TUNEL staining was performed to evaluate cell death. Hepatocyte inflammation was assessed by liquid chromatography-mass spectrometry (LC-MS) measurement of various ceramides in an independent cohort consisting of DCD (n = 13) and DBD (n = 10) allografts. The means of IHC findings were compared with a Student's *t*-test, and Spearman's test was used to determine significant correlations with respect to donor and recipient clinical parameters. For ceramide analysis, univariate non-parametric Wilcoxon sign-rank test was performed.
Results: Prior to transplantation, DBD livers have higher levels of leukocyte infiltration and increased expression of pro-apoptotic factor FasL and high levels of intracellular adhesion molecule-1 (ICAM-1), as compared to DCD allografts. Following reperfusion, the neutrophil infiltration and platelet deposition associated with

I/R injury remained more prominent in DBD grafts as compared to their DCD counterparts. Further, there was greater upregulation of pro-inflammatory ceramides in post-transfusion biopsies in DBD as compared to DCD allografts. Despite decreased inflammation, DCD allografts had significantly higher levels of cell death than DBD grafts, which correlated with the duration of warm ischemia time as well as significantly higher levels of aspartate aminotransferase (AST) in the serum of recipients of DCD livers in the acute post-transplant period.
Conclusions: These data suggest that ischemia/reperfusion injury causes a non-inflammatory necrosis in DCD allografts with an appreciable effect on early graft function. The long-term consequences of increased inflammation in DBD allografts and cell death in DCD allografts are unknown and warrant further investigation.

P0033
PROSPECTIVE EVALUATION OF INTRAOPERATIVE PORTO-CAVAL GRADIENT AFTER MAJOR HEPATECTOMY

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Background and Aims: It has been shown that portal pressure may be a predictor of posthepatectomy liver failure. The aim of this study was to prospectively assess the relationship between porto-caval gradient (PCG) and portal vein pressure (PVP) with respect to postoperative morbidity and mortality.
Methods: From January 2013 to June 2014, measurements of caval and portal pressure at the end of a major liver resection have been performed in a single institution. Preoperative, intraoperative and postoperative data have been prospectively collected in the e-Hpbchir database. The main endpoint was the severe morbidity (Dindo Clavien score grade III-V). Secondary endpoints were the comprehensive complication index, postoperative ascitis and jaundice.
Results: A total of 119 patients have been included. Median value (range) of PCG and PVP were 6 mmHg (0–22) and 13 mmHg (3–29), respectively. The optimal cutoff value that best predicts severe morbidity was 12 mmHg for PCG (AUC=0.657) and 18 mmHg for PVP (AUC=0.664). Severe morbidity occurred more often in patients with PCG ≥12 mmHg (70% vs 25%; $P = 0.0001$) and in those with PVP ≥18 mmHg (69% vs 23%; $P < 0.0001$). The PCG correlates the PVP ($r = 0.79$; $P < 0.001$). Mean comprehensive complication index was significantly higher in patients with elevated PCG [43 (±29) vs 22 (±20); $P = 0.005$] and elevated PVP [42 (±26) vs 21 (±20); $P = 0.0004$]. Patients with PCG ≥12 mmHg developed more often postoperative ascitis and jaundice compared to those with PCG <12 mmHg (35% vs 3%; $P < 0.0001$). Similar differences were observed in patients with a PP ≥18 mmHg (27% vs 3%; $P = 0.0006$).
Conclusions: This study prospectively validates the predictive value of porto-caval gradient for severe morbidity after major hepatectomy, similar to that we observed with portal vein pressure retrospectively. This confirms that portal flow modulation must be explored to improve post-operative results of major hepatectomy.

P0034
GOOD OUTCOME OF PATIENTS TRANSPLANTED WITH MELD SCORE GREATER THAN 30: PAUL-BROUSSE HOSPITAL EXPERIENCE

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Background and Aims: Several studies observed that patients transplanted with MELD score ≥30 have a lower survival after liver transplantation (LT) as compared to patients with less severe disease but this issue remains controversial. Such information is crucial to evaluate sickest candidates for LT. To compare survival rates between patients with MELD ≥30 and MELD <30 at time of LT.
Methods: This is a single centre, retrospective study, performed from 2008 to 2014. Patients with fulminant hepatitis and combined liver-kidney transplantation were excluded. We compared survival of patients with MELD score ≥30 to that of patients with MELD score <30, transplanted in the same period in the same centre.
Results: We included 106 patients with MELD ≥30 at time of LT, who had the following characteristics (medians): age 53.2 years (95% CI 50.8–55.2), INR 4.22 (3.8–4.8), bilirubin 22.4 mg/dl (17.8–26.2), creatinine 0.99 mg/dl (0.85–1.3), albumin 30 g/l (29–31), MELD 36 (34–37). Median hospital stay before LT was 22 days. 8.5% were HIV-positive, 25.5% were hospitalised in ICU on the day of LT, 4.7% had renal replacement therapy, 8.5% required mechanical ventilation and 7.5% vasopressors. Indication of LT was cirrhosis in 86.75%, HCC 5.7% and retransplantation for 7.55% of patients. Patients with MELD score ≥30 had a median hospital stay after LT of 33 days. Three-year survival did not differ significantly between patients with MELD <30 ($n = 561$) and ≥30 (78.7±2.1% vs. 83.8±4.2% $p = 0.45$). Similar 3-year survival was also observed if the population is divided according to a MELD cut-off of 35: 80.7% (MELD ≥35) vs. 79.4% (MELD <35), $p = 0.9$. In the MELD ≥30 group, survival at 3 years of HIV-positive patients was 0% versus 92.1±3.3% in non-HIV patients ($p < 0.001$). Patients hospitalized in ICU had the same survival at 3 years than those who were not in ICU: 77.8 vs. 85.3%, $p = 0.4$. In patients with MELD ≥30, patients who died within 3 years had a more prolonged hospital stay before LT than survivors (43 vs. 21 days, $p = 0.001$), longer hospital stay after LT (61 vs. 30 days, $p < 0.001$) and were younger (48 vs. 54 years, $p = 0.04$; probably related to the HIV indication). All other tested parameters did not differ between patients who died and survivors. Prognostic scores such as SOFA, CLIF-SOFA and IGS2 were not helpful in predicting survival.
Conclusions: Liver transplantation in patients with MELD ≥35 is safe with excellent survival at 3 years. HIV infection is a major pejorative predictor of death after liver transplantation in our cohort

P0035
ARE THERE DEMOGRAPHIC, BEHAVIOURAL AND PSYCHOSOCIAL FACTORS THAT PREDICT ALCOHOL CONSUMPTION BEFORE OR AFTER LIVER TRANSPLANTATION?

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Background and Aims: Alcohol related liver disease (ALD) is the second commonest indication for liver transplant in the UK. Selection of these patients is controversial due to the perceived

self-inflicted nature of the disease and due to the risk of relapse to alcohol post-transplantation. It is important to be able to identify those patients who are at risk of relapse in order to provide support.
To determine whether the Relative Risk Factors for Relapse (RRFR) score and/or High Risk Alcohol Relapse scale (HRAR) can predict alcohol consumption in pre and/or post-liver transplant patients.
Methods: Retrospective analysis of patients' medical records. Univariate analysis was conducted to compare categorical variables between the relapse versus the non-relapse group.
Results: Between September 2008 and March 2013, 197 patients were listed for a liver transplant with a diagnosis of ALD. At the time of analysis 114 of the 197 patients (57.9%) had received a liver transplant. Thirty-two (16.3%) had been removed due to deterioration or death, twenty-four (12.2%) had improved and no longer required a transplant and sixteen patients (8.1%) were removed for consuming alcohol. Five of the patients were excluded from post-transplant analysis due to death before 90 days. Fourteen patients were reported as relapsing to alcohol post-transplant. The HRAR scale failed to predict alcohol consumption in patients pre-transplant ($P = 0.454$) or post-transplant ($P = 0.218$). None of the three variables that make up the HRAR scale were predictive of relapse. A high RRFR score was highly predictive of relapse in pre-transplant patients ($P = 0.004$) but not post-transplant ($P = 0.6$). Of the seven variables that make up the RRFR score illicit drug misuse was the only predictor of pre-transplant alcohol consumption ($P = 0.005$). Poor social support and lack of replacement strategies were found to be predictive of relapse in post-transplant patients ($P = 0.016$ and 0.044 respectively).
Conclusions: The HRAR and RRFR are currently used in four of the seven UK liver transplant centres. As the HRAR is not predictive of alcohol consumption its use to assist in decision making regarding transplant suitability should be discontinued. The RRFR is a predictor to alcohol consumption pre-transplant however further investigation is required to validate its use in the transplant setting.

P0037
PROGRESSIVE PORTAL AND LOBULAR FIBROSIS IN LONG TERM SURVIVING PEDIATRIC LIVER GRAFTS: DIFFERENT COMPARTMENTS WITH DIFFERENT BACKGROUNDS

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Background and Aims: Long-term survival after pediatric liver transplantation (LTx) has improved. However, multiple studies have shown that long-term graft survival is associated with increasing histological abnormalities, predominantly fibrosis and hepatitis. We previously reported the presence of graft fibrosis in 2 studies of CSA treated patients at 1 and 10 years after LTx. The aim of this study was to evaluate graft fibrosis according to the acinar distribution and establish a correlation with clinical characteristics of patients who all received tacrolimus as the main immunosuppressive therapy. We hypothesize that the portal, perisinusoidal and centrilobular distribution of graft fibrosis results from different underlying conditions.
Methods: We reviewed the histological features in protocol liver biopsies taken at 1 and 5 years after transplantation of 47 children. Fibrosis was assessed according to the Liver Allograft Fibrosis Scoring system (LAFSc).
Results: Fibrosis was present in 84% of the 1-year biopsies and 86% of the 5-year biopsies. An increased incidence of all 3 types of fibrosis was observed between 1 and 5 years. At 5 years centrilobular fibrosis was present in 85% of cases, sinusoidal fibrosis in 79% and portal fibrosis in 62%. The number of biopsies that showed histological hepatitis or minimal reactive changes decreased between 1 and 5 years after transplantation and were not related to fibrosis. There was a trend toward an association between biliary complications and portal fibrosis at 5 years ($p = 0.06$) while total bilirubin and γGT were clearly associated with portal fibrosis ($p = 0.02$ for both). Other liver function tests were not related to fibrosis. Centrilobular fibrosis was related to HLA mismatches ($p = 0.05$), primarily at the HLA class I level. Rejection was related to the development of sinusoidal fibrosis ($p = 0.02$). Previously described relation between fibrosis and (pre)transplant related factors, e.g. CMV status, cold ischemia time, donor age and graft type could not be confirmed in this study.
Conclusions: Using the LAFS scoring system, we found in this new cohort of tacrolimus treated patients a high incidence of progressive fibrosis in the 1 year and 5 year protocol biopsies after LTx. Progression of fibrosis was observed in all acinar compartments and each of the 3 locations is associated with different clinical conditions. Portal fibrosis with biliary complications, centrilobular fibrosis with HLA class I mismatch and sinusoidal fibrosis with previous rejection episodes.

P0038
CHARACTERIZATION OF HUMORAL REJECTION IN LIVER PEDIATRIC TRANSPLANTATION

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Background and Aims: The aim of the study is to correlate C4d immunostaining, pathological features of liver grafts and the presence of donor specific antibodies (DSA) in pediatric transplant recipients.
Methods: 31 graft biopsies (27 ten-year biopsies and 5 biopsies on indication) performed in 31 pediatric transplant recipients were reviewed for histological features according to Banff recommandations. Immunostaining for C4d was performed on deparaffinized tissue sections and analyzed semiquantitatively. DSA were quantified by Luminex Single Antigen.
Results: C4d immunostaining was positive in 28/31 biopsies with the following pattern: portal stroma (diffuse 7/31, focal 13/31), portal vein endothelial cells (7/31, 23%), peribiliary capillar plexus (18/31, 58%), centrilobular vein endothelial cells (10/31, 32%) and sinusoidal cells (19/31, 69%). 11/31 biopsies were classified as chronic rejection, defined by centrilobular fibrosis with hepatocellular loss. Diffuse portal stroma and endothelial (at least one endothelial type) C4d immunostaining were significantly associated with histological features of chronic rejection ($p = 0.0036$ and 0.013). DSA results were available in 20 patients: 20% had DSA class 1, 75% DSA class 2. 30% were negative. C4d immunostaining of sinusoidal cells ($p = 0.0018$) and of at least one type of endothelium ($p = 0.024$) was significantly associated with DSA. There was no significant association but only a trend for chronic rejection and DSA ($p = 0.069$).
Conclusions: The C4d immunostaining of portal stroma and endothelial cells is correlated on the one hand with the pathological features of chronic rejection, on the other hand with the presence of DSA. These results strongly suggest the participation of humoral mechanisms in liver graft chronic rejection in pediatric population.

P0039

PREDICTION OF HCC RECURRENCE AFTER LIVER TRANSPLANTATION BASED ON EXPLANTS PATHOLOGY: COMPARISON OF PREDICTION ACCURACY OF 4 PROGNOSTIC MODELS IN A PROSPECTIVE EXTERNAL COHORT

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Background and Aims: After liver transplantation for HCC, histological features of the explant liver are of great importance to identify patients at high risk of recurrence. This is a crucial step to adapt screening strategies, and to select best candidates to changes in immunosuppression and potential adjuvant therapies. The aim of this study was to test the performances of published models of prediction of HCC recurrence based on histo-pathological findings in an external cohort of liver transplant patients.
Methods: Among the 5 published models, one included the presence of giant/bizarre cells, a variable which is not available in usual pathological reports of liver explants and was therefore excluded (Parfitt et al. 2007). The 4 other models (Iwatsuki et al. 2000, Chan et al. 2008, Mazzafero et al. 2009, Decaens et al. 2011) used a mix of usual variables (number of nodules, size of the largest, tumor differentiation, existence of micro/macrovascular invasion, tumor burden uni or bilobar) and were tested in a cohort of 318 patients transplanted in 20 French centers for HCC between 2003 and 2005 and followed prospectively for 5 yrs.
Results: Predicting performances of the different models are reported in table 1. All 4 explanted models were able to identify patients with tumours features associated with a 5-yr rate of recurrence >50% ($p < 0.001$), compared to low risk pts in all models (see table). However, predictive accuracy of US models was lower than expected in this European cohort compared to European models. In particular, low risk score in the Chan model was associated with a 24% risk of recurrence in the current validation cohort and very high risk score in the Iwatsuki model was associated with a lower risk of recurrence of 50% in the French cohort. By contrast, expected predictive values of both French and up to seven models were found reproducible.
Conclusions: Both French (Decaens et al.) and Up to seven models may serve as a tool for identification of explant-based prediction of patients at high risk of recurrence in a European population and therefore guide change in immunosuppression and identification of best candidates for adjuvant therapies.

Table (abstract P0039).

Study	Participants	Model components	Performance of models in original reports	5Y recurrence rate in the current French cohort
Decaens et al. 2011	Training cohort (TC) n = 373 Validation cohort (VC) n = 345	Tumor differentiation Largest size Number	**0 to 5 points for each variable** **score <4** 20.8±2.8% (TC) and 6.3±2.6 (VC) **score ≥4** 40.0±5.4% (TC) and 35±10.7 (VC)	**score <4** 14.5±2.8% **score ≥4** 51.6±4.6% ($p < 0.05$) $P < 0.001$
Mazzaferro et al. 2009	n = 1556	Largest size Number Microvascular invasion	**Within up-to-seven (largest size + number) & no microvascular invasion** 9.1 (5.6–14.5)% **Beyond Up-to-seven & microvascular invasion** 39.9 (30.8–51.7)%	**Within up-to-seven & no microvascular invasion** 10.8±2.2% ($p < 0.001$) **Beyond Up-to-seven & microvascular invasion** 54.5±5.4% $P < 0.001$
Chan et al. 2008	Test cohort n = 116 Validation cohort n = 72	Largest size Bilobar Macrovascular invasion Non-well-diff.	**Predicting cancer recurrence score (PCRS)** PCRS ≤0 (n = 76): 0% PCRS 1/2 (n = 31): 19.4% PCRS ≥3 (n = 9): 66.7%	PCRS ≤0: 24.0±2.9% PCRS 1/2: 38.6±7.6% PCRS ≥3: 61.8±8.8% $P < 0.001$
Iwatsuki et al. 2000	n = 344	Bilobar location Largest size cm Vascular invasion (micro/macro)	**Prognostic risk score (3.1–15.0 points for each)** <7.5 pts: 0% ≤11.0 pts: 9% <15.0 pts: 60% ≥15.0 pts: 95%	<7.5 pts: 15.5±2.8% ≤11.0 pts: 34.6±4.9% <15.0 pts: 48.7±9.7% ≥15.0 pts: 50.4±8.6% $P < 0.001$

P0040

LONG-TERM CARDIOVASCULAR MORTALITY AFTER LIVER TRANSPLANTATION: ANALYSIS OF RISK FACTORS

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Background and Aims: Cardiovascular (CV) diseases are a major cause of death after liver transplantation (LT) at long term. The objective of this study was to analyse the main causes of mortality and the risk factors for death and for cardiovascular death 5 years after LT.
Methods: Data from 1819 liver receptors followed-up in 15 Spanish centres were collected prospectively. The main causes of death 5 years after LT have been analysed. Multivariate COX regression model analysis was used to identify baseline variables related to 5-years mortality and CV mortality.
Results: Among the 1819 patients the overall 5-year mortality was 26.3% (n = 479). The main causes of death were liver diseases (27.1%), cancer (20.1%), infectious diseases (16.3%) and cardiovascular diseases (12.1%). The multivariate analysis identified the following baseline predictive factors for 5-year mortality: age (HR 1.01, 95% CI 1.00–1.02; p 0.025), hepatocellular carcinoma (HR 1.26, 95% CI 1.02–1.5; p 0.027), hepatitis C virus infection (HR 1.52, 95% CI

1.23–1.87; p 0.000), hyperlipidaemia (HR 0.5, 95% CI 0.38–0.87; p 0.021), insulin-dependent diabetes (HR 1.62, 95% CI 1.26–2.07; p 0.000), hyperuricemia (HR 1.35, 95% CI 1.06–1.73; p 0.015), chronic renal failure (HR 1.45, 95% CI 1.12–1.88; p 0.004) and previous CV events before LT (HR 1.58, 95% CI 1.17–2.14; p 0.003). The age at the time of transplantation (HR 1.033, 95% CI 1.0–1.06; p 0.033), chronic renal failure (HR 2.31, 95% CI 1.25–4.27), previous CV events (HR 2.3, 95% CI 1.123–4.71; p 0.023), hypercholesterolemia (HR 0.13; 95% CI 0.019–0.9; p 0.048) and the use of mycophenolate mofetil (MMF)-free immunosuppressive regimen (HR 2.36; 95% CI 1.23–4.51; p 0.009) were identified as the main predictors for CV mortality at 5 years.

Conclusions: CV diseases are the fourth cause of death among LT recipients after 5 years of follow-up. The age at the time of transplantation, the presence of previous CV disease, patients with hypercholesterolemia, chronic renal failure and patients treated with MMF-free regimen showed an increased risk of CV death.

P0041

THE IMPACT OF DONOR AND RECIPIENTS SINGLE NUCLEOTIDE POLYMORPHISMS OF LIVING FIBROSIS PROGRESSION IN LIVING LIVER TRANSPLANTATION FOR HEPATITIS C

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Background and Aims: Recently, some previous reports showed that some of single nucleotide polymorphism (SNPs) affect liver fibrosis progression in patients with hepatitis C virus (HCV). In this study, we examined the impact of donor and recipient SNPs on the progression of fibrois after liver transplantation for HCV.

Methods: This cohort study enrolled 42 patients with HCV who underwent liver transplantation in our hospital. We genotyped 3 genotypes (rs4374383, rs2629751, rs9380516) that emerged in GWA study of liver fibrosis progression related to HCV infection (Gastroenterology 2012; 143:1244–1252 e1241–1212), rs8099917 in IL28B that were associated interferon treatment response in Japanese (Nat Genet 2009; 41: 1105–1109), and rs738409 in PNPLA3 that were identified as a genetic predictor for hepatic stetosis (Hepatology 2010; 52: 1274–1280) and recently were reported as significant predictor of fibrosis with HCV, using a Taqman assay. One year progress of liver fibrosis was defined as the development of fibrosis (≥F1) at liver biopsies 1 year after liver transplantation. The association with one year progress was examined. The time to ≥shininuyma F2 (portoportal septa) or HCV-related mortality was also analyzed.

Results: Results; Patients with fibrosis (≥F1) of liver at one year after liver transplantation were detected in 30 cases (70%). The rs2629751 genotype have showed significantly association with fibrosis progression at one year after liver transplantation (AA: GG or GA = 57%: 88%, P < 0.05). A total of 21 patients developed the primary outcome of ≥F2 (portoportal septa) or HCV-related mortality. The time to ≥stage 2 fibrosis or HCV related mortality have showed significantly difference in only donor's rs2629751 genotype (AA: GG or GA = 5.5±0.6 years: 3.6±0.7 years, p = 0.025).

Conclusions: The rs2629751 genotype is an important predictor of posttranspant outcome in HCV. This result might be useful in donor selection for liver transplantation with HCV, and may guide decisions regarding early antiviral treatments.

P0042

SAFETY AND EFFICACY OF SOFOSBUVIR AND SIMEPREVIR TREATMENT IN HEPATITIS C GENOTYPE 1 LIVER TRANSPLANT RECIPIENTS WITH ADVANCED FIBROSIS/RECURRENT CIRRHOSIS

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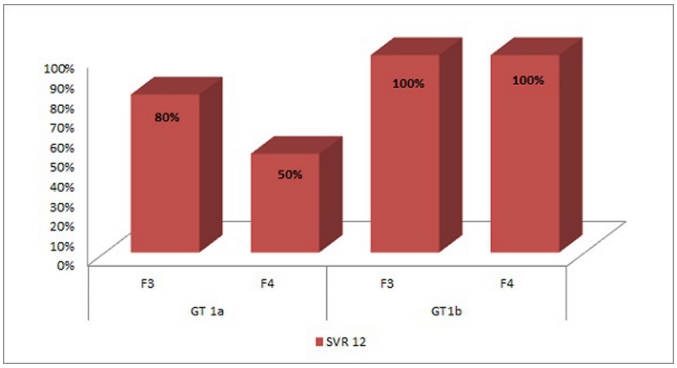
Background and Aims: Recurrent hepatitis c (HCV) is invariable in liver transplant recipients (LTR) which can result in accelerated fibrosis and graft failure. There is limited data with sofosbuvir and simeprevir treatment (COSMOS regimen) in HCV positive LTR with advanced fibrosis. We aim to submit our safety and efficacy data of COSMOS regimen in HCV genotype 1 LTR with advanced fibrosis or cirrhosis.

Methods: Retrospective analysis of 19 LTR with recurrent HCV genotype 1 with 53% METAVIR F4 were treated with sofosbuvir 400 mg orally daily and simeprevir 150 mg orally daily for 12 weeks at the Miami Transplant Institute. There were 9 LT recipients with HCV genotype 1a (4 cases with F4) and 10 with HCV genotype 1b (6 cases with F4). The median time from LT to start of treatment was 65 months (range 2–194). The majority of individuals (84%) had failed previous treatment with interferon-based regimens and 21% were treated with regimens containing a protease inhibitor. This study was an IRB approved study.

Results: All 19 individuals completed 12 weeks of antiviral therapy with sofosbuvir and simeprevir and only one interrupted therapy for 1 week due to hospitalization for problems unrelated to hepatic decompensation or drug-related adverse events (AEs). No severe AEs occurred; the most common AEs were fatigue (21%), headache (16%), nausea (16%), skin rash and photosensitivity (11%). All individuals had undetectable HCV-RNA at EOT. SVR12 was achieved in 84% of individuals: 100% in HCV genotype 1b and 67% in HCV genotype 1a (P = 0.046).

Conclusions: COSMOS treatment for 12 weeks with sofosbuvir and simeprevir was well tolerated in LTR with advanced fibrosis and cirrhosis. Overall SVR12 rate was 84% with distinct difference among genotype 1a and 1b, 67% and 100% respectively. The effect of longer duration of therapy or addition of ribavirin in LTR with advanced fibrosis/cirrhosis and HCV genotype 1a infection needs to be further evaluated.



P0043

RATE OF EMPLOYMENT AFTER LIVER TRANSPLANTATION IN FRANCE: A SINGLE CENTRE STUDY

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Background and Aims: A return to gainful employment is an important outcome parameter after liver transplantation (LT). A

recent study in the United States (US) has shown a very high rate of unemployment after LT (75%). To date, no data is available in France, where the public health insurance program guaranties financial protection for everyone.
Aims: To assess employment rate after LT in a French LT centre and to determine factors associated with employment after LT.
Methods: All patients transplanted in our centre between January 2000 and April 2011 were subjected to a questionnaire, provided they met the following criteria: (1) aged 18–65 at time of LT; (2) being alive 1 year after LT, (3) being alive, not retired and discharged from hospital at time of survey, (4) being French resident and affiliated to French national health insurance.
Results: 345 LT were performed in 314 patients during the study period. 109 patients were not considered for the study: 23 patients died within the first year after LT, 28 patients were dead at the time of the survey, 3 were still hospitalized, 7 patients were living in a foreign country, 11 were retired and 37 had an age >65 after LT. 205 patients were included in the study. The response rate was 76.6% (157/205): mean delay after LT: 6.1±0.9 years, French nationality 77.7%, male gender 73.2%, mean age at LT 48.8±9.9 yr. Aetiologies of liver disease were the following: alcohol 32.5%, hepatitis C 26.1%, alcohol and hepatitis C 3.8%, hepatitis B 15.3 %, biliary cirrhosis 5.1%, auto-immune 2.5%, other causes 14.7%. At 2-year, 43.3% of patients were employed. The demographic variables associated with post LT employment included male gender ($p < 10^{-3}$), aged under 40 at time of LT ($p = 0.02$), sedentary work occupation ($p = 0.007$), raising children younger than 18 years old at time of LT ($p = 0.01$), high level of education ($p = 0.001$), not being affiliated to the French universal health coverage so called 'CMU' ($p = 0.001$). Among patients who did not return to work after LT, only 53.3% stated they felt physical disabilities.
Conclusions: The rate of going back to work after LT in France was 43.1%, which was higher than the one reported the US study. However this rate remains low and encouraging policies are needed in order to help liver recipients who wish to work after LT.

P0044
MRCP TAKEN ONE MONTH AFTER LDLT MAY PREDICT BILIARY COMPLICATION
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Background and Aims: Biliary complications are common and important complications after living donor liver transplantation (LDLT). There are several diagnostic tools for detection of biliary complications. Magnetic resonance cholangiopancreatography (MRCP) is known to be an accurate method in evaluation of the biliary system. However, the value of MRCP findings to predict biliary complication after liver transplantation have not been studied. Therefore, we have studied the value MRCP imaging taken one month after LDLT for prediction of biliary complications.
Methods: We included 201 patients who took LDLT from 2006 to 2010. Patients took MRCP 1 months after LDLT. MRCP images were analyzed for angle of anastomosis in 3D image, presence of filling defect, length of filling defect in 3D image and MIP image, presence of intrahepatic bile duct dilatation, biliary stricture and leakage. Angle of anastomosis was defined as angle between donor site bile duct and recipient's common bile duct. Patients who received biliary intervention during the follow up period were considered to have biliary complication.
Results: During a follow up until December 31, 2012, 94 patients developed biliary complication. In multivariate analysis using Cox-regression method, decreased angle of anastomosis (hazard ratio, 0.986; 95% CI, 0.979 to 0.993; P = 0.000), filling defect in MIP image (hazard ratio, 1.838; 95% CI, 1.178 to 2.868; P = 0.007) and leakage (hazard ratio, 3.457; 95% CI, 1.215 to 9.835; P = 0.020) were significant risk factors for development of biliary complication. Cut-off value for angle of anastomosis was 109.85° with 68.1% sensitivity and 79.2% specificity.
Conclusions: In MRCP images taken 1months after LDLT, decreased angle of anastomosis as well as filling defect in MIP image and biliary leakage, is associated with increased risk of biliary complications.

P0045
LONG TERM LOW DOSE STEROID TRIPLE IMMUNOSUPPRESSIVE REGIMENS IN POST TRANSPLANT AUTOIMMUNE HEPATITIS (AIH) PATIENTS REDUCES THE INCIDENCE OF RECURRENT DISEASE AND IS NOT ASSOCIATED WITH INCREASED MORTALITY
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Background and Aims: Optimal immunosuppression for post transplant AIH patients reduces the risk of AIH recurrence (AIHR) while minimizing the complications associated with long term use. Our practice is to maintain long-term low dose steroids in addition to at least one other immunosuppression. We describe our experience with a focus on factors associated with AIHR as well as graft and patient survival over a 15 year period.
Methods: We collected data on patients transplanted between Dec 1999 – Sep 2014 in a single transplant unit. AIHR was diagnosed by the presence of elevated IgG, consistent histology and exclusion of other causes. Outcomes of AIHR, regraft-free and overall survival were assessed using Kaplan–Meier survival analysis methods and log-rank tests. All analyses were performed using IBM SPSS Statistics 22 with $p < 0.05$ deemed to be statistically significant.
Results: 78 patients were transplanted with a median follow-up of 101 months. The cohort was mainly Caucasian (78%), female (77%), with type 1 AIH (91%) and a mean age of 42.7 years (SD=14.3). Patients were transplanted for acute liver failure in 14% of the cohort and cirrhosis in 86%. Only 5 patients developed AIHR, giving estimated rates of 0%, 5% and 11% at 1, 5 at 10 years respectively. Overall survival was 92%, 86% and 72%, and re-graft free survival was 85%, 74% and 64% at 1, 5 at 10 years respectively.
Maintenance immunosuppression data were available for 67/78 patients. 11 patients were censored as they had either died or had follow up <1 year post transplant. Table 1 documents the different regimens and outcomes. 12 patients were not on prednisolone (PR) for reasons of PTLD (1), steroid related side effects (4), patient choice (1) and records were unavailable for the remainder (6). 63% of patients continued on triple immunosuppression with PR without any significant deleterious effects on mortality, graft survival or AIHR.
Regraft-free survival was significantly shorter in AIH type 2 than in type 1, with rates at 5 years of 38% and 78% respectively ($p = 0.02$), mainly due to incidences of early post transplant hepatic artery thrombosis. Overall survival was similar in the two groups ($p = 0.24$).
Conclusions: This is the largest single centre cohort of post transplant AIH patients. Type 1 AIH patients have better early outcomes than type 2 patients but similar overall survival. Triple

immunosuppression with low dose PR seems to be generally safe and leads to lower incidences of AIHR than in the published literature.

Table 1. Different immunosuppression combinations and associated outcomes

Drug combination	%	Overall survival (%)		Re-graft free survival (%)		Freedom from AIHR (%)	
		1 year	5 year	1 year	5 year	3 year	5 year
T only	5	100	100	100	67	100	100
T + A/M	14	100	89	78	78	100	100
PR + T	18	100	100	82	82	100	100
PR + T + A/M/S	63	100	91	100	83	94	91
		p value 0.626		p value 0.959		p value 0.311	

Survival rates are based on Kaplan–Meier estimates, and include only those patients who have survived past 1 year from transplant.
A, azathioprine; M, mycophenolate mofetil; PR, prednisolone; S, sirolimus; T, tacrolimus.

P0046
RESPONSE TO LOCO-ABLATIVE TREATMENT OF HCC PRIOR TO LIVER TRANSPLANTATION IS ASSOCIATED WITH LOW RECURRENCE RATES IN PATIENTS EXCEEDING THE MILAN CRITERIA

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Background and Aims: The Milan criteria are the most widely accepted selection criteria for liver transplantation (LT) in cirrhotic patients with hepatocellular carcinoma (HCC). Several groups have challenged these criteria and shown that tumors exceeding MC can be transplanted. However, these attempts have been associated with an increased rate of HCC recurrence. The aim of this study was to investigate if response to loco-ablative bridging therapy before LT can be used to identify patients exceeding the MC who can undergo LT without an increased HCC recurrence risk.
Methods: Patients who underwent LT for HCC at our institution between 2002 and 2013 were included. Baseline CT scans and scans prior to LT were independently reviewed by two radiologists to assess MC and response to loco-ablative therapies (TACE, RFA) according to mRECIST. A multivariate regression model was used to assess risk factors for HCC recurrence.
Results: One hundred seventy-four HCC patients (89% male, mean age 60 years) underwent LT. Fatty liver cirrhosis (55%) and chronic hepatitis C (30%) were the most common underlying liver diseases. Seventy-one patients (41%) exceeded MC (up to 9 lesions) and ablative treatment was performed in the vast majority of patients (94%). Response to loco-ablative bridging therapy was not associated with HCC recurrence in patients within the MC. Patients exceeding MC but achieving complete remission after loco-ablative bridging therapy had a comparable 5-year recurrence rate to all patients within MC (16.7% vs. 8.5%; $p = 0.588$). In contrast, 5-year recurrence rate was significantly higher in patients outside the criteria and stable disease/partial response (21.9%; $p = 0.024$) and in those with progressive disease (48.1%; $p < 0.001$). Tumor grading, histologic and radiologic evidence of vascular invasion were independent risk factors for HCC recurrence in a multivariate regression analysis. Overall survival was independently associated with radiologic evidence of vascular invasion and response to ablative treatment with a five-year post LT survival of 75% in the overall cohort.
Conclusions: Response to neoadjuvant loco-ablative treatment identifies a subgroup of patients exceeding the MC that can undergo LT without an increased risk of HCC recurrence. From our findings we conclude that LT can be offered to selected patients with HCC tumor stage beyond the MC.

P0047
RECIPIENT (BUT NOT DONOR) ADIPONECTIN POLYMORPHISMS ARE ASSOCIATED WITH POST TRANSPLANT HEPATIC STEATOSIS

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Background and Aims: Metabolic syndrome and hepatitis C genotype 3 infection are known predictors of post-transplant steatosis but the role of genetic factors are less well established. Adiponectin is an adipokine with anti-inflammatory and anti-atherogeneic properties. Hypoadiponectinemia is well described in pre-transplant NAFLD independent of insulin resistance and is implicated in the loss of hepatic fat in 'burnt out' NASH cirrhosis. The role of Adiponectin in the liver transplant recipient is unclear. We explored the association between donors and recipients Adiponectin genetic polymorphisms and biopsy proven post-transplant hepatic steatosis.
Methods: We identified consecutive patients transplanted for HCV cirrhosis between 2006–2011 at a tertiary care center in the United States. This was a case-control study with cases defined as subjects with steatosis (5% or greater) and controls with minimal or no steatosis (<5%) on post transplant biopsy. Donors and recipients were tested for the rs1501299, rs2241766, rs266729 and rs2241766 Adiponectin polymorphisms using the QLAamp DNA Mini Kit assay testing by TaqMan SNP genotyping assay.

Table: Recipient and donor adiponectin polymorphisms and post-transplant steatosis

Factor	Overall (N = 111)		No steatosis (N = 72)		Steatosis (N = 39)		p-value [a]
	N	Summary	n	Summary	n	Summary	
Recipient rs1501299	110		71		39		**0.028**
GG		55 (50.0)		30 (42.3)		25 (64.1)	
non-GG		55 (50.0)		41 (57.7)		14 (35.9)	
Donor rs1501299	111		72		39		0.19
GG		59 (53.2)		35 (48.6)		24 (61.5)	
non-GG		52 (46.8)		37 (51.4)		15 (38.5)	
Recipient rs2241766	111		72		39		0.83
TT		87 (78.4)		56 (77.8)		31 (79.5)	
non-TT		24 (21.6)		16 (22.2)		8 (20.5)	
Donor rs2241766	106		69		37		0.61
TT		80 (75.5)		51 (73.9)		29 (78.4)	
non-TT		26 (24.5)		18 (26.1)		8 (21.6)	
Recipient rs266729	109		71		38		**0.008**
CC		75 (68.8)		55 (77.5)		20 (52.6)	
non-CC		34 (31.2)		16 (22.5)		18 (47.4)	
Donor rs266729	111		72		39		0.22
CC		71 (64.0)		49 (68.1)		22 (56.4)	
non-CC		40 (36.0)		23 (31.9)		17 (43.6)	
Recipient rs17300539	108		72		36		**0.026**
GG		94 (87.0)		59 (81.9)		35 (97.2)	
non-GG		14 (13.0)		13 (18.1)		1 (2.8)	
Donor rs17300539	109		71		38		0.55
GG		92 (84.4)		61 (85.9)		31 (81.6)	
non-GG		17 (15.6)		10 (14.1)		7 (18.4)	

Values are presented as Mean±SD, Median [P25, P75] or N (column %).
[a] From Pearson's chi-square test.

Results: Clinical data were collected for a total of 302 patients who were transplanted for HCV during the study period. 111 patients who had available donor DNA, recipient DNA, and a post-transplant liver biopsy were included in the analysis. Of these,

35% developed significant steatosis (cases). Cases and controls were well matched in terms of age, gender, donor risk index, cold ischemia time as well as pre and post transplant metabolic syndrome. Cases were more likely to be white and to have genotype 2. Univariable analysis showed that recipient rs1501299, rs266729 and rs17300539 polymorphisms were found to be associated with post-LT steatosis. After adjusting for race and hepatitis C genotype, recipients with GG rs1501299 were 2.5 times more likely to develop steatosis after LT than those with non-GG genotypes. Similarly, recipients with GG rs17300539 genotype were more likely to develop steatosis after LT than those with non-GG genotypes ($p = 0.049$). None of the donor Adiponectin polymorphisms were associated with post transplant steatosis.

Conclusions: Recipient but not donor Adiponectin gene polymorphisms are associated with post-transplant hepatic steatosis. Further studies are needed to explore the potential role for Adiponectin in the pathogenesis of post transplant steatosis and NASH.

P0048

RISK FACTORS FOR EARLY KIDNEY DYSFUNCTION AFTER LIVER TRANSPLANTATION AND THEIR IMPACT ON GRAFT LOSS: INSIGHTS FROM THE LIVER MATCH COHORT STUDY

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Background and Aims: Early Kidney Dysfunction (EKD) is a frequent event during the first six months after liver transplantation (LT) and can be a determinant of long-term outcome. On the other hand, the introduction of MELD to prioritize candidates for LT has resulted in a greater propensity to transplant those with poor kidney function. The impact of this policy on the risk of EKD and its effect on 5-year graft loss were the objectives of this study.

Methods: We evaluated 1302 patients undergoing LT in Italy from June 1, 2007 to May 31, 2009 belonging to the Liver Match cohort (median donor and recipient age: 56 and 55 years, respectively, males: 79%; HCV+ patients: 45.5%). EKD was staged based on the lowest estimated glomerular filtration rate (eGFR, MDRD-4 formula) at 3 and 6 months after LT, as follows: eGFR >60 ml/min (EKD stage 1–2); 45 ≤ eGFR < 60 ml/min (EKD stage 3a); 30 ≤ eGFR < 45 ml/min (EKD stage 3b); and eGFR <30 ml/min (EKD stage 4–5).

Results: We identified 812 patients as EKD stage 1–2 (62.4%), 404 as EKD stage 3a/b (31.0%) and 86 as EKD stage 4–5 (6.6%). Multinomial logistic analysis identified four recipient risk factors for EKD: old age, female gender, and low eGFR and sodium levels at LT. Odds ratios and 95% CI for EKD stage 3a/b vs stage 1–2 were as follows: age (×10 years): 1.63, 1.40–1.89; female vs male gender: 2.29, 1.68–3.12; *e*GFR at LT (30–60 vs >60 ml/min): 2.55, 1.79–3.64; *e*GFR at LT (<30 vs >60 ml/min): 2.82, 1.35–5.89; serum sodium at LT (per 5 meq/L): 0.86, 075–098. Odds ratios and 95% CI for EKD stage 4–5 *vs* stage 1–2 were as follows: age (×10 years): 1.70, 1.28–2.27; female *vs* male gender: 2.0, 1.58–3.46; *e*GFR at LT (30–60 vs >60 ml/min): 2.72, 1.50–4.94; *e*GFR at LT (<30 vs >60 ml/min): 9.74, 4.08–23.28; serum sodium at LT (per 5 meq/L): 0.73, 0.58–0.92.

Cox regression identified EKD stage as a significant risk factor for 5-year graft loss, after adjusting for the other significant predictors (donor age, recipient HBcAb and HCV positivity). Assuming EKD stage 1–2 as reference level, the hazard ratios of graft loss for EKD stages 3a, 3b, and 4–5 were 0.89 ($p = 0.4729$), 1.68 ($p = 0.0059$) and 3.38 ($p < 0.0001$), respectively.

Conclusions: The development of EKD stage ≥2b entails a high risk of graft loss after LT and that the probability of EKD can be estimated before LT. This suggests that high risk patients can be identified and should be considered for alternative treatment strategies, including combined liver-kidney transplantation.

P0049

EVALUATION OF ACOUSTIC RADIATION FORCE IMPULSE IMAGING (ARFI) ELASTOGRAPHY FOR NON-INVASIVE LIVER FIBROSIS ASSESSMENT IN PATIENTS AFTER LIVER TRANSPLANTATION

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Background and Aims: Transient elastography proved to accurately diagnose graft fibrosis and liver cirrhosis in patients with recurrent HCV after liver transplantation. The aim of this prospective study was to evaluate the diagnostic accuracy of liver stiffness measurement (LSM) by ARFI elastography staging graft fibrosis in liver transplant recipients undergoing protocol liver biopsy.

Methods: 108 liver transplant recipients (59% males; mean age 52±12 y) were enrolled in the study. Main etiologies that led to liver transplantation were viral hepatitis (n = 37), alcoholic liver disease (n = 18) acute liver failure (n = 10) and genetic liver diseases (n = 9). All patients received ARFI elastography of the liver at the same day of the protocol biopsy. Biopsy results with rejection activity index (RAI) ≥5 were excluded from the study (n = 7). Liver histology was used as reference. All laboratory and clinical data were recorded.

Results: The area under the curve (AUC) of LSM detecting ≥F2, ≥F3 and F4 were 0.75, 0.86 and 0.98, respectively, in comparison to liver biopsy. Cut off values of LS were 1.48 m/s, 1.62 m/s and 1.99 m/s, respectively. The sensitivity and specificity were 82% and 70%, 100% and 81%, 100% and 97%, respectively. Although there was a significant correlation between LS and histologic liver fibrosis ($r = 0.759$, $p < 0.001$) and hepatic activity index (HAI) ($r = 0.342$, $p < 0.001$), there was no correlation detected with RAI ($r = -0.118$, $p = 0.239$).

Conclusions: ARFI elastography is able to diagnose liver cirrhosis with an excellent diagnostic accuracy in liver transplant patients. Interestingly, cut off values were higher than the previously described LS values staging liver fibrosis in non-transplanted patients with chronic liver diseases.

P0050

NEUROLOGICAL SYNDROME 'DE NOVO' IN FAMILIAL AMYLOIDOTIC POLYNEUROPATHY AFTER LIVER TRANSPLANT

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Background and Aims: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a progressive neurodegenerative, disabling and life-threatening polyneuropathy affecting the peripheral and autonomic nerves, first identified in Portugal, with a wide variation in age of onset and symptoms. TTR is synthesized mainly in the liver, and liver transplant (LT) seems to have a favorable effect on the course of neuropathy, being a potential treatment currently available for TTR-FAP. Some patients developed transitory "de novo" neurological symptoms (unilateral paresthesias, aphasia, dysarthria, dizziness and vertigo syndrome), all of them 8 or more years after LT.

Methods: Medical records from TTR-FAP adult patients submitted to liver transplant in a single center more than eight years ago were reviewed (until December 2006, 681 LT were performed, 179 by FAP; 125 patients are still alive, 28 of them with neurological syndrome *de novo*). All the patients were submitted to a thorough neurological workup, and almost all had normal exams. MRIs were not performed due to protocol cardiac pacemaker insertion prior to liver transplant.
Results: 28 patients (22%) developed the same transitory, sporadic "de novo" neurological symptoms such as generalized absence, drowsiness, headache, unilateral paresthesias, hemiparesis, aphasia, dysarthria, slurred speech, double vision, blurred vision, partial seizures and myoclonic, dizziness and vertigo syndrome, behavior changes and mental confusion. All patients are treated or have been in the past with calcineurin inhibitors (CNI), 26 with cyclosporine and 2 with tacrolimus; those in which CNI were suspended improved or remained without these symptoms.
Antiepileptic drugs were prescribed with partial remission of the symptoms.
Conclusions: Neurological syndrome *de novo* have a significant impact on quality of life of these patients. These symptoms may be due to the natural progression of the disease apparently due to the increased survival of these patients with the LT.
We must review the immunosuppression of these patients.
Further studies are needed to improve treatment and quality of life of the transplanted TTR-FAP.

P0051
PERIOPERATIVE WHITE BLOOD CELL COUNT AS A MARKER FOR PATIENT AND GRAFT SURVIVAL AFTER ORTHOTOPIC LIVER TRANSPLANTATION

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Background and Aims: Orthotopic liver transplantation (OLT) is a standard procedure in endstage liver disease. However, recipients of OLT have an 10–15% risk to die within one year after transplantation. We evaluated whether different parameters of infection including cytokines and blood cells predict post-OLT mortality, graft survival and rate of acute rejection.
Methods: We collected clinical and laboratory data of 104 patients undergoing liver transplantation between 2011 and 2012 from Hannover Medical School. Patients were stratified by (i) patient survival, (ii) graft survival and (iii) episode of rejection(s) and were followed from OLT for one year. Laboratory data of peritransplant period (0–4 days after OLT) were analysed.
Results: Inflammatory markers like CRP and procalcitonin had no significant effect on one-year patient or graft survival after OLT ($p = 0.3$ or $p = 0.8$ respectively). Interestingly, white blood cell count (WBC) early after OLT was a prognostic marker for patients ($p = 0.019$) and graft survivial ($p = 0.03$). Importantly, white blood cell count early after OLT was independent from rate of acute rejection episodes. White blood cell count >20,000/μl within the first four days after OLT was associated with a higher patient and graft mortality. Patient mortality was 30% (WBC >20,000/μl) in comparison to 13% (WBC <20,000/μl). These results were independent from the underlying liver disease or type of immunosuppressive regimen.
Conclusions: These data demonstrate that white blood cell count >20,000/μl early after OLT is a cheap prognostic marker for patient and graft survival, while perioperative procalcitonin and CRP have no influence.

P0052
INFUSION OF THIRD-PARTY MESENCHYMAL STROMAL CELLS AFTER LIVER TRANSPLANTATION: A PHASE I, OPEN-LABEL, CLINICAL STUDY

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Background and Aims: Mesenchymal stromal cells (MSC) are multipotent bone marrow progenitors that have demonstrated significant immunosuppressive effects in various *in vivo* and *in vitro* studies. This study aimed to be the first evaluation of the safety and tolerability of MSC infusion after liver transplantation in a prospective, controlled phase-1 study.
Methods: Clinical grade MSCs were locally collected from the bone marrow of unrelated healthy donors. They were cultured in a GMP-compliant lab, underwent extensive quality controls and were frozen for storage in a MSC bank. When needed for patient treatment, MSC were thawed and intravenously injected into patients. 10 liver transplant recipients under standard immunosuppression (TAC-MMF-low dose steroids until day 30) received $1.5–3 \times 10^6$/kg MSC on post-operative day 3 ± 2. These patients were prospectively compared to a group of 10 control (MSC-) liver recipients. Primary endpoints were MSC infusion toxicity, and incidence of cancer and opportunistic infections at month 6. Secondary endpoints were patient and graft survivals and rejection at month 6, as well as the effects of MSC on recipients' immune function and on immunohistology of at month 6 graft biopsies.
Results: No MSC infusional toxicity was observed. Both groups were comparable in terms of donor and recipient characteristics. There was no difference in primary end-points between control and MSC groups. No patient developed *de novo* cancer. There was no statistical difference in patient and graft survivals or in rejection rates. There was no graft rejection in the MSC group. Month-6 graft biopsies were not different according to Banff and fibrosis scores.
Conclusions: This phase 1 study showed excellent tolerability and safety of a single infusion of third-party MSC after liver transplantation. There were no graft safety issues and no excess of immunosuppression after MSC injection. Further analyses of consequences of MSC injection on the immune profile are needed. The possibility of avoiding calcineurin-inhibitors with repeated MSC injections as main immunosuppressive therapy and/of tolerance induction by MSC infusion should be investigated by further studies.

P0053
DINSTICT INTRAHEPATIC CYTOKINE PROFILES FOR THE DIFFERENTIATON OF ACUTE CELLULAR REJECTION VS RECURRENT HEPATITIS C IN LIVER TRANSPLANTED PATIENTS

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Background and Aims: Graft reinfection in liver transplanted patients with hepatitis C virus (HCV) infection is universal and triggers rejection episodes as well as liver damage and fibrosis. Reliable differentiation between HCV infection early after liver transplantation and acute cellular rejection (ACR) is of great importance for clinicians and poses a challenge for pathologists.

The aim of this study was a comprehensive analysis of intrahepatic cytokine and chemokine profiles and identification of inflammatory signatures that could be used to differentiate ACR from recurrent HCV in liver transplant recipients.
Methods: 60 HCV liver transplanted patients that underwent liver biopsy because of elevated transaminases in the first year after liver transplantation were included in this study. RNA was extracted from embedded biopsies and the expression of 47 chemokines and cytokines (mRNA), involved in inflammatory response, was determined by quantitative real time PCR.
Receiver operating (ROC) analysis was performed for all measured parameters and correlations in in the expression of cytokines and chemokines were analysed with SAS 9.3 and R 3.1.0.
Results: Blinded pathological reading revealed a definite diagnosis in 20 biopsies (10 ACR and 10 graft HCV, "training cohort"), a probable diagnosis in 21 patients (8 recurrent HCV and 13 ACR) and no conclusive result in 19 biopsies. Expression levels of five cytokines had an AUC >0.7 regarding diagnosis of an ACR in the training cohort. Their cut off values were estimated using Youden-Index and the calculation of odds ratio with 95% CI, revealed 3 parameters (CCL2, CXCL9, CCL11) that were differently expressed in the two patient groups (ACR vs graft hepatitis C). Distinct expression pattern revealed an up regulation of CCL2 together with CCL4, CCL5, HGF, IL18, TGFb1 and PDGF in patients with ACR that was not observed in those with HCV recurrence. Similarly, CXCL9 correlated with IFNg expression in patients with ACR but with CCL4 in HCV graft hepatitis.
Importantly, the identified parameters and expression patterns did not differentiate ACR and recurrent HCV hepatitis in the group with probable pathological diagnosis.
Conclusions: ACR and HCV-induced graft hepatitis in liver transplant recipients show distinct intrahepatic cytokine and chemokine patterns. This work shows that the histopathological analysis is equivocal and that further analysis of intahepatic cytokines and chemokines signatures could complement und facilitate the histopathological diagnostics.

P0054
SAFETY AND EFFICACY OF SUBCUTANEOUS HEPATITIS B IMMUNOPROPHYLAXIS USING "ON DEMAND" APPROACH AND COST ANALYSIS: A SINGLE CENTER EXPERIENCE

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Background and Aims: Hepatitis B immunoglobulin (HBIG) administration is the backbone for prophylaxis of HBV re-infection after liver transplantation (LT). Long term effects and efficacy of HBIG are well known only for intravenous (IV) and intramuscular (IM) formulations. Aim is to investigate efficacy, safety and feasibility of "on demand" subcutaneous (SC) administration of the new formulation of HBIG BT088 (Zutectra®) in LT patients.
Methods: A total of 47 pts (11F, 36M, mean age 60±9 years) were switched from IV (13 pts – 32%) or IM (28 pts – 68%) to SC HBIG administration during a period of 3 years from January 2011. The conversion to SC administration occurred at a mean time of 36 (±38) months from the LT. They were prospectively enrolled and followed up for at least 38(±12) months. The dose of HBIG was initially standardized to 1000 IU/week SC. After a period of stabilization, patients were offered a "on demand" approach, aiming at a targeted level of serum antiHBs ranging between 100 and 150 UI/L.
Results: All patients were HBV-DNA negative at the time of transplantation (9 spontaneously – 19%, 38 under NUCs – 81%) and were on a combination prophylaxis with HBIG and NUCs. All patients became rapidly independent for the weekly SC self-injection. The treatment was effective in maintaining trough anti-HBs levels greater than 100 UI/l in all patients. 90% of patients showed a mean HBsAb titer greater than 136 UI/l. Overall, mean values of HBsAb were 239 UI/l (±115). The mean HBsAb titer prior to switching to SC formulation was 384±125, with a mean monthly injection of 5000 U/L. In 60% of the patients the administration of SC HBIg were at least on a Q2-weeks administration (Table 1). No drug-related side effects or injection site problems were observed. As anticipated, the anti HBs titers progressively decreased with time from OLT but remaining above the "safe" level of 100 U/L.
Conclusions: SC HBIG for long term prophylaxis of post LT HBV re-infection has proven to be safe, well-accepted and effective in maintaining the targeted protective anti-HBs levels. Moreover, individualization of immunoprophylaxis according to the lowest protective anti-HBs titers is easily applicable with the SC formulation, allowing the exploration of new schedules in order to improve costs per patient/year while maintaining efficacy.

P0055
SAFETY AND EFFICACY OF ANTICOAGULATION THERAPY FOR PORTAL/SPLANCHNIC VEIN THROMBOSIS IN PATIENTS WITH LIVER CIRRHOSIS ON THE WAITING LIST FOR LIVER TRANSPLANTATION

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Background and Aims: In patients with advanced cirrhosis, the presence of portal vein thrombosis (PVT) represents a cause of increased morbidity and mortality. Despite the high frequency of PVT in patients with end stage liver disease on the waiting list (WL) for liver transplantation (LT), there are few data on the efficacy and safety of anticoagulation therapy in this setting. Therefore neither clear recommendations, nor consensus regarding the optimal regimen and duration of anticoagulation therapy have been addressed in recent consensus publications on this specific issue.
Aim: To investigate the safety and efficacy of anticoagulation therapy for portal/splanchnic vein thrombosis in patients with liver cirrhosis on the WL for LT.
Methods: We included 121 patients with liver cirrhosis included on the waiting list for LT.
Results: The prevalence of PVT on the National WL for LT is 19.1%. Out of 121 patients, 44.6% received anticoagulant therapy. 35.1% received low weight heparine and 64.9% received acenocumarol for a mean time of 9.4±1.4 months. 39.6% of them had recanalization of the PVT, while 31.4% remained stable after 3 months of anticoagulant therapy. Complete and partial repermeabilization was acheived in 14.5% (7/48) and 85.5% (41/48) respectively. 13.2% of patients were transplanted. Overall death was encountered in 28.1% (34/121) of patients while on the WL and 4.9% (6/121) died after LT. Severe hemmorhagic events (variceal bleeding/hemoperitoneum/ hemorrhagic stroke) occured in 9.2% of patients receiving anticoagulant therapy.
Conclusions: PVT prevalence on WL for LT is high. Anticoagulant therapy is administred in 44.6% of patients, is safe and rather efficacious in recanalization of PVT before LT.

P0056

THE IMPACT OF METABOLIC SYNDROME AND PREVALENT LIVER DISEASE ON LIVING DONOR LIVER TRANSPLANTATION: A PRESSING NEED TO EXPAND THE POOL

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Background and Aims: Organ shortage has been the ongoing obstacle to expand liver transplantation world-wide. Living donor liver transplantation (LDLT) was hoped to improve this shortage. The aim of the present study was to analyse the impact of metabolic syndrome and prevalent liver disease on living donations. of new strategies.
Methods: From July 2007 to December 2012, a total of 171 deceased donor liver transplants and 190 living donor liver transplants (LDLT) were performed at our institution. In total, 265 patient were excluded because of either diabetes or a mody mass index (BMI) >28. Eight hundred potential living donors were assessed according to a step-wise evaluation protocol. The age of the donors receiving work-up ranged from 18 to 60 years. The majority were first and second degree relatives of the patients.
Results: Only 190 (24%) were accepted for donation and 610 (76%) were rejected. Some potential donors were excluded at initial screening due to incompatible blood groups (115; 14.3%), social reasons (40; 5%), or elevated liver enzymes (9; 1%). A total of 85 (10.5%) donors were excluded due to positive hepatitis serology. Steatosis resulted in the exclusion of 84 (10.5%) donors. In addition, 80 (10%) potential donors were rejected due to variations in biliary anatomy, and 20 (2%) were rejected due to aberrant vascular anatomy. Rejection due to biliary-related aberrancy decreased significantly in the second half of our program (14.5% vs. 5%, p = 0.0001). In total, 110 (13.7%) potential donors were rejected due to insufficient remnant volume (<30%) as determined by CT volumetry, whereas 24 (3%) were rejected due to a graft-to-recipient body weight ratio less than 0.8%.
Conclusions: Metabolic syndrome and viral hepatitis negatively impacted our living donor pool. Expanding the donor pool requires the implementation of new strategies.

P0057

BACTERIOBILIA IN LIVER TRANSPLANTED PATIENTS

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Background and Aims: Liver transplanted patients are at increased risk for bacteriobilia, as they undergo biliary tract handling, immunosupression and frequent antimicrobial therapy. Bile microbial patterns seem to differ from non-transplanted patients.
We aimed to characterize biliary microbial isolates retrieved during endoscopic retrograde cholangiopancreatography (ERCP) to liver transplanted patients.
Methods: Validated microbial isolates, and respective resistance testing, from cultures of bile and biliary prosthesis retrieved endoscopically from liver transplanted patients at our centre between June 2009 and December 2013 were retrospectively analyzed. Sensitivity to usual empirical antibiotics for cholangitis was assessed; as well as for vancomycin in *Enterococci* isolates. Extended spectrum beta-lactamase (ESBL) expressing bacteria were considered resistant to piperacillin/tazobactam, independently of *in vitro* sensitivity.
Results: A total of 328 different isolates were cultured from samples retrieved during 229 ERCP performed to 86 patients, 69.8% males, median age 54 years [19;71].
The majority of isolates (57.3%, n = 188) were gram negative bacteria, 40.5% (n = 133) gram positive bacteria and 2.1% (n = 7) fungi.
Considering only bacterial isolates, gram positive predominated in inaugural *vs* subsequent ERCP (63.0% *vs* 39.4%, p = 0.18). Differences were not significant among other subgroups, where gram negative isolates were more common (patient gender, biliary stenosis, lithiasis, prosthesis, and time since liver transplant).
Among gram negatives, 86.7% were *Enterobacteriaceae* (47.2% of them *Escherichia coli*), 7.4% *Pseudomonas aeruginosa* and 6.7% *Acinetobacter baumanii*. Among gram positives, 65.6% were *Enterococci* (43.0% of them *E. faecium*), 12.0% *Streptococci* and 5.3% *Staphylococci*. All isolated fungi were *Candida* species.
Resistance rates in *Enterobacteriaceae* were 45.3% to ciprofloxacin, 25.8% to cefotaxime, 23.3% to piperacillin/tazobactam and 1.8% to meropenem; 57.7% were multi-drug resistant. All *Acinetobacter* and 41.7% of *Pseudomonas aeruginosa* isolates were resistant to ciprofloxacin. Among *Enterococci*, 17.8% were resistant to vancomycin, and 36.4% resistant to ampicillin (10 confirmed *E. faecium* were sensible to ampicillin).
5.6% and 1.6% of bile and prosthesis cultures, respectively, were sterile.
Conclusions: Considerable levels of antimicrobial resistance were found. Empiric antibiotic therapy may be adjusted to inaugural vs subsequent ERCP.

P0058

SOFOSBUVIR-BASED ANTIVIRAL THERAPY IS HIGHLY EFFECTIVE IN RECURRENT HEPATITIS C IN LIVER TRANSPLANT RECIPIENTS: A "REAL-LIFE" CANADIAN MULTICENTER EXPERIENCE

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Background and Aims: Hepatitis C (HCV) recurrence is universal in patients who are viremic at the time of liver transplant (LT), and adversely affects outcomes with compromised patient and graft survival. This study aims to evaluate the efficacy, safety and tolerability of regimens containing sofosbuvir (SOF) in the treatment of HCV recurrence in all genotypes (GT) in patients outside of clinical trials in all seven Canadian transplant centers.
Methods: 120 LT recipients from across Canada with HCV recurrence were started on SOF based regimens [SOF + simeprevir (SIM) +/− ribavirin (RBV) n = 53; SOF + pegylated interferon + RBV n = 25; SOF + RBV n = 36; and SOF + Ledipasvir n = 6] between January and November 2014. Patient mean age was 58±6.85 years, and the majority (83%) were GT1, male (81%), Caucasian (85%) and treatment experienced (82%). 30% had fibrosing cholestasic (FCH)/aggressive HCV in the graft, 50% had F3/4 fibrosis. 58% were on tacrolimus, 36% on cyclosporine, and 6% on rapamycin. Median time from LT to treatment was 27 (1–

309) months. Two patients were HIV co-infected. The primary outcomes include patient and graft survival, on- and end-of-treatment response and sustained virological response at 12 weeks after treatment end (SVR12), and adverse events (AEs), including rejection, need for blood transfusions or growth factors, and other complications.

Results: 54/57 (95%) patients were HCV RNA undetectable at end of treatment (EOT), and SVR12 was achieved in 24/27 (89%) patients, with 3 relapsers. Week 4 of treatment data was available in 2/3 of patients and 58% had HCV RNA levels below the lower limit of quantification (LLQ). Creatinine levels (umol/L) were unchanged [median pre-treatment: 112 (54–257) vs. on-treatment: 109 (57–400), p = NS]. Severe anemia (Hb <85 g/l) requiring erythropoietin and/or blood transfusion occurred in 13% of patients, more frequently in RBV based regimens. Generalized AEs included fatigue, headache and nausea (seen in 20%), and photosensitivity skin rash (3%). 7 patients died (n = 1 intracranial bleed, n = 4 sepsis and n = 2 decompensated). No episodes of rejection occurred on treatment and no interactions with any concomitant immunosuppressive agents were reported.

Conclusions: Sofosbuvir based antiviral therapy for HCV recurrence after LT is well tolerated, and preliminary results suggest high efficacy overall, including in aggressive HCV and cirrhotic patients. No immunosuppression changes were needed when using these regimens.

P0059

META-ANALYSIS: SVR12 WITH SIMEPREVIR AND SOFOSBUVIR (SMV+SOF) FOR 12 WEEKS IN 225 LIVER TRANSPLANT (LT) RECIPIENTS WITH HCV GENOTYPE 1 (HCV-1)

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Background and Aims: Recently, the all-oral combination of SIM+SOF became available for the treatment of HCV-1 patients; however, safety, efficacy, and effectiveness data with SIM+SOF in post-LT patients is limited due heterogeneous study designs and small sample sizes. Our goal was to perform a meta-analysis of the available literature for SIM+SOF with and without ribavirin (RBV) in post-LT patients.

Methods: In November 2014, we conducted a systematic literature search for 'simeprevir' in MEDLINE, AASLD, APASL, DDW, EASL, and World Transplant Congress for the year of 2014. Literature search review and data extraction were performed independently by two authors (NN and BY) using a case report form and checked by a third author (MN) with discrepancies resolved by consensus. Inclusion criteria were: original studies of ≥5 post-LT HCV-1 patients treated with SIM+SOF±RBV that had SVR12 data. Exclusion criteria were HIV or HBV co-infection. We used random-effects models to estimate effect sizes, and Cochrane Q-test (*p*-value <0.10) with I^2 statistic (>50%) to assess study heterogeneity.

Results: Our analysis included eight studies and a total of 225 post-LT patients with SVR12 data. The majority of studies were conducted in a university setting (n = 6). Most patients were male (range 59–81%). Proportion with advanced fibrosis ranged from 6 to 41%. Pooled rates were as follows: end of treatment response was 98% (CI: 95.1–99.1%); SVR4 was 90% (CI: 82–94.6%); and SVR12 was 85% (CI: 79.4–89.3%). SVR 12 data was available in 2 studies to compare patients with mild fibrosis (n = 69) to those with advanced fibrosis (n = 24). SVR12 in patients with mild fibrosis was 90.1% (CI: 61.8–98.1%), which was significantly higher than SVR12 in advanced fibrosis at 55% (CI: 13.9–90.6%), OR 8.4 (CI: 2.2–31.74, p = 0.002). The most common pooled side effects were: fatigue 22% (47/216), headache 7% (n = 17/208), and pruritus 7% (n = 17/171). No treatment withdrawals were reported in these studies. Data was insufficient to examine SVR12 in subsets of patients treated with RBV vs. without and in patients with prior treatment failure vs. treatment-naïve patients.

Table 1. Characteristics of studies included in meta-analysis

First author, year	Setting	Collaboration	ITT analysis	Sample size with SVR12 data	Age (years)	Male, N (%)	Advanced fibrosis, N (%)
Aquel B, 2014	University	Multi-center	Yes	66	61.6±6	77 (76)	28 (28)
Gordon FD, 2014	University	Single center	No	15	–	–	1 (6)
Gutierrez JA, 2014	University	Single center	No	37	60.2±9.3	22 (59)	14 (38)
Suliman I, 2014	Community	Single center	Yes	8	62.5	6 (60)	–
Lutchman GA, 2014	University	Single center	Yes	31	62.3±5	43 (81)	11 (33)
Nair S, 2014	University	Single center	Yes	27	56±5	28 (61)	19 (41)
Ripper SJ, 2014	Community	Single center	No	23	60.9	21 (84)	7 (28)
Crittenden N, 2014	University	Single center	No	18	–	–	–

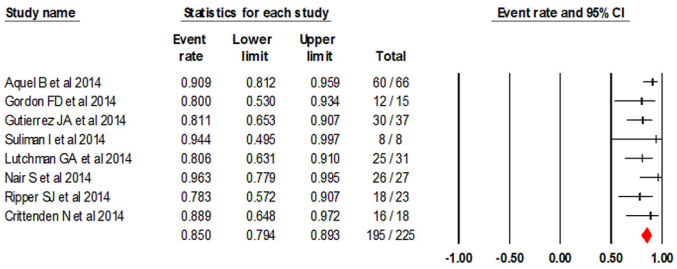

Figure 1. Pooled estimate of SVR12 in post-liver transplant patients.

Conclusions: SVR12 rates with SIM+SOF±RBV for 12 weeks in post-LT patients was approximately 85% suggesting high effectiveness even in this special population. This combination also appeared well-tolerated in the post-transplant setting. Patients with mild fibrosis are more likely to achieve SVR12.

P0060

ANTIVIRAL THERAPY WITH SOFOSBUVIR PLUS RIBAVIRIN IN HCV-INFECTED CIRRHOTIC PATIENTS AWAITING LIVER TRANSPLANTATION: PRELIMINARY DATA FROM A SINGLE-CENTRE EXPERIENCE

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Background and Aims: Clearance of HCV infection before liver transplantation (LT) prevents recurrence after LT and could improve graft survival. We evaluated safety and efficacy of all oral antiviral therapy (Tx) with sofosbuvir (SOF) plus ribavirin (RBV) in HCV-infected cirrhotic patients (pts) before LT.

Methods: Among pts awaiting LT in our Centre, since June 2014 to date we enrolled 24 HCV cirrhotic pts with HCC within Milan criteria (15 pts) and/or decompensated liver disease. Median MELD 13 (range 7–19); Child-Turcotte-Pugh score B 71% and C 17%; 83% male, mean age 55.4 years, mean BMI 25.8. Mean baseline HCV RNA 6.17 $\log_{10}$ IU/mL (4.91–7.11 $\log_{10}$ IU/mL); GT1b 55%, GT1a 4%, GT2 4%, GT3 29%, GT4 8%; IL28 CC 20%; 63% had previous HCV Tx. Planned treatment is SOF 400 mg/die (compassionate use) and RBV (weight-based: 1000 mg daily if <75 kg; 1200 mg daily if ≥75 kg) for 48 weeks or until LT.

Results: To date, 10/24 pts received 12 weeks of Tx. Antiviral treatment resulted in rapid suppression of circulating virus with a median decrease in HCV RNA of 3.27 $\log_{10}$ IU/mL after 1 week of Tx; 10/20 pts (50%) at week 4 and 14/14 (100%) at week 8 were HCV RNA negative. Other results available until now are

depicted in the Table. No serious adverse events were observed. RBV reduction and/or epoetin administration was required in 25% of pts; 3 pts underwent blood transfusion and 3 were hospitalized due to complications of liver disease. Of the 7 pts transplanted until now, 4 had HCV RNA <15 IU/mL for a median of 62 days (31–91) and discontinued antiviral Tx at the time of LT. All those 4 pts are currently HCV RNA negative after a median follow-up post-LT of 45 days (16–69). Two pts underwent LT while still being HCV RNA positive after less than 4 weeks of Tx, and the last one was transplanted 3 days ago having HCV RNA <15 IU/mL since 5 days.

Time point (No. of patients)	HCV RNA negative	HCV RNA <15 IU/mL	MELD score, median (range)	Child–Pugh score	
				B (%)	C (%)
Basal (N = 24)	0%	0%	13 (7–19)	71%	17%
Week 4 (N = 20)	50%	75%	14 (6–23)	33%	39%
Week 8 (N = 14)	100%	100%	13 (7–18)	64%	21%
Week 12 (N = 10)	100%	100%	14 (7–21)	40%	30%
Week 16 (N = 7)	100%	100%	15 (9–21)	29%	20%
Week 4 after LT (N = 3)	100%	100%			

Conclusions: An all oral Tx with SOF plus RBV induces rapid HCV clearance and is well tolerated in cirrhotic pts awaiting LT. Preliminary post-LT SVR4 rate is promising in pts undergoing LT after at least 1 month from viral clearance. Further data will be presented as they become available.

P0061

TREATMENT WITH NEW ALL-ORAL DIRECT ACTING ANTIVIRALS IN HCV-RECURRENCE IN LIVER TRANSPLANT-SETTING INDUCES A REDUCTION OF IMMUNOSUPPRESSIVE DRUG LEVELS

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Background and Aims: *Introduction:* New direct acting antiviral agents (DAA) are an important advance in HCV treatment. DAA combinations have shown high efficacy in difficult-to-treat patients. They are not supposed to have significant pharmacological interaction with immunosuppressants (IS), so they are good candidates to treat liver transplant (LT) patients.
Aim: To describe efficacy and safety of DAA-treatments in patients with HCV-recurrence after LT. We focused our study in the potential immunosuppressant level reduction during DAA-treatment.
Methods: We evaluated 71 LT patients under IFN-free DAA treatment, including 18 patients with fibrosis cholestatic hepatitis. We monitored them weekly the first month and then every 2 weeks.
Results: 53 males. Patients' mean age was 56 years old (range 41–74); Range time from LT 1–198 months. Genotype distribution: 1a, 8 patients; 1b, 55, and 5 genotype 3 and 3 patients genotype 4. Fibrosis evaluation of HCV recurrence (METAVIR): F <2, 23 patients; F3, 10; F4, 31. 52 treatment experienced. All patients were on a stable IS regimen: 50 tacrolimus, 12 cyclosporine and 9 with mTOR.
Efficacy: 46/71 achieved RVR; and 58/70 achieved RNA undetectable at week 8, and 60/60 at week 12. Patients did not developed any significant adverse events. 2 deaths were reported, not related to antiviral treatment.
Mean IS level reduction was 30% (range 3–82%) between week 1 and 26 (mean 6). IS doses were adjusted in 42/65; 6/68 had an acute rejection.
The majority of patients improved AST levels. 13/71 improved albumin level; 35/71 improved bilirubin level; and 21/71 improved INR. Almost all patients improved non-invasive fibrosis scores by week 4: APRI decreased a mean 3.5 (range 0.2–10.2), FORNS mean 1.8 (range 1.2–10.2), Fibroindex mean 0.9 (range 0.1–5.6) and Fib-4 mean 4.4 (range 0.7–19.5). IS level reduction correlates significantly with non-invasive scores improvement: Pearson values for APRI 0.41 ($p = 0.005$); FORNS 0.55 ($p < 0.0001$); Fibroindex 0.45 ($p = 0.05$) and Fib-4 0.32 ($p = 0.038$). 37/71 patients improved MELD score between 1 and 17 points.
Conclusions: All-oral DAA combinations have a high viral efficacy with an excellent safety profile in LT patients. Despite the absence of a pharmacological interaction we have observed a decrease in immunosuppression levels, this could be related to a quick liver function recovery that improves IS clearance. In these patients, it would be recommended to closely monitor immunosuppression levels.

P0062

CHANGES IN CEREBRAL HEMODYNAMICS IN CIRRHOTIC PATIENTS AFTER LIVER TRANSPLANTATION

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Background and Aims: Changes in cerebral hemodynamics before and during liver transplantation (LT) have been related to poor neurological outcomes after LT. Improvement in cognitive function after LT has been related to decreased cerebral white matter lesions induced by microvascular lesions (leukoaraiosis) secondary to low-grade cerebral edema. However most of studies have addressed these changes in the acute setting during and immediately after LT in cirrhosis as well as in acute liver failure and few have evaluated the long-term changes in cerebral hemodynamics in this population, therefore we aimed to investigate the long-term changes on cerebral hemodynamics in cirrhotic patients after LT.
Methods: This was a prospective cohort study. Patients were evaluated by Transcranial Doppler Ultrasonography (TCD) obtaining Pulsatility index (PI) a marker of cerebrovascular structural integrity and breath-holding index (BHI) a marker of cerebrovascular reactivity which were measured in the middle cerebral artery pre and post-LT. Critical flicker frequency (CFF), psychometric hepatic encephalopathy score (PHES) and West-Haven criteria were used for HE characterization. Mean follow up after LT was 6 months.
Results: We included 17 patients (11 males); median age pre-LT was 45 (40–53) years, major etiology was hepatitis C virus (8/17), most of patients were Child–Pugh B (12/17), MELD score was 15.5 (12–17.5), MELD-Na 18, PHES −3 and CFF 38.5 Hz. Previous to the LT 11/17 patients had HE (6/4/1; covert, grade I and grade II HE, respectively), 5/17 ascites and 5/17 hepatocellular carcinoma. A decrease in PI was observed in all patients after LT and an increase in BHI in 9/17 (PI 1.01 and BHI 0.77 pre-LT vs PI 0.78 and BHI 0.72 post-LT, $P < 0.001$ and 0.740, respectively). The change on PI corresponds to a decrease of 22% and an increase on BHI of 55% after LT. Clinical improvement in cognition was observed in all patients with overt HE after LT.
Conclusions: These results show an improvement in cerebral hemodynamics at long-term after LT in cirrhosis, indicating less arterial cerebral vasoconstriction (decrease in PI) and an improvement in cerebrovascular autoregulation in 9/17 patients (increase in BHI). This could explain the improvement in cognitive function after LT and the decrease in cerebral white matter lesions induced by microvascular lesions described in some studies. TCD

could be considered as a useful tool for the assessment of the changes in cerebral hemodynamics pre and post-LT in cirrhosis.

P0063

SOFOSBUVIR PLUS RIBAVIRIN FOR THE TREATMENT OF SEVERE HCV RECURRENCE AFTER LIVER TRANSPLANTATION: PRELIMINARY DATA FROM A SINGLE-CENTRE EXPERIENCE

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Background and Aims: Recurrent hepatitis occurs in the vast majority of HCV-positive recipients who are viremic at the time of liver transplantation (LT). We evaluated safety and efficacy of all oral antiviral therapy (Tx) with sofosbuvir (SOF) plus ribavirin (RBV) in a population of HCV-infected patients (pts) having severe recurrent hepatitis after LT.
Methods: Among HCV viremic pts transplanted in our Centre since 2000, we enrolled to date 107 pts affected by HCV RNA-positive recurrent hepatitis with fibrosis score F3 (21 pts) or F4 (86 pts) according to Metavir. 78% male, mean age 60.5 years, mean BMI 24.7; 49% are receiving cyclosporine, 43% mycophenolate, 38% tacrolimus and 3% m-TOR inhibitors. GT1 75% (GT1a 13%), GT2 5%, GT3 15%, GT4 5%; IL28 CC 24%; 65% had prior HCV Tx. According to compassionate use, pts receive SOF 400 mg/die plus RBV (weight-based: 1000 mg daily if <75 kg; 1200 mg daily if ≥75 kg) for 24 weeks. Median time from LT to Tx 4.96 years (0.25–14.25); mean baseline HCV RNA 6.62 $\log_{10}$ IU/mL (3.4–7.9 $\log_{10}$ IU/mL); mean GFR 76 mL/min.

Time point (No. of patients)	HCV RNA negative	HCV RNA <15 IU/mL	Mean Hb (g/dL)	Mean GFR (mL/min)	MELD score, median (range)	Child score	
						B (%)	C (%)
Basal (N = 107)	0%	0%	12.8	76	12 (6–22)	26%	5%
Week 4 (N = 98)	51%	88%	11.1	75	12 (6–26)	22%	6%
Week 8 (N = 77)	99%	100%	10.2	75	12 (6–22)	23%	6%
Week 12 (N = 31)	100%	100%	10.8	75	13 (6–28)	29%	13%
Week 16 (N = 9)	100%	100%	10.5	87	8 (6–18)	44%	11%
Week 24 (N = 2)	100%	100%	11.4	101	12 and 14	A6 and B7	
Week 4 after end of Tx (N = 2)	50%	50%	12.6	102	11 and 16	A5 and B9	

Hb, haemoglobin.

Results: Until now, 31/107 pts completed 12 weeks of Tx. After 1 week of Tx, median decrease in HCV RNA was 3.61 $\log_{10}$ IU/mL; 50/98 (51%) at week 4 and 76/77 (99%) at week 8 were HCV RNA negative. Other results available until now are shown in the Table. Of the 2 pts who completed the planned therapy and reached 4 weeks post-Tx, one relapsed; he is IL28 TT, GT1a and the only one who tested HCV RNA <15 IU/mL at week 8 on Tx. The most common adverse events were fatigue, headache and nausea. During Tx, 1 patient had variceal bleeding (day 78) and 1 died due to multiorgan failure (day 86); 25% received blood transfusion or epoetin; 1 patient temporally discontinued RBV because of skin rash. Minimal immunosuppression dose adjustments were required on Tx and no rejection episodes were recorded.
Conclusions: In pts with severe HCV recurrence post-LT, an all oral antiviral regimen using SOF plus RBV is very well tolerated and easy to manage, while allowing rapid HCV clearance. Data on sustained virologic response will be presented as they become available.

P0064

RIBAVIRIN PRE-TREATMENT IN LIVER TRANSPLANT PATIENTS WITH HCV RECURRENCE: RESULTS FROM PEARL MULTICENTRE RANDOMIZED TRIAL

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Background and Aims: The treatment of liver transplanted patients with HCV recurrence is still a critical issue as the antiviral treatment regime based on the new DAA is not universally available and, due to high cost, restricted to patients with moderate/severe fibrosis (F≥3). In a previous pilot uncontrolled study we showed that 8-week ribavirin (RBV) pre-treatment before standard antiviral therapy with RBV and PEG-IFN, exhibits a better safety profile and therapy outcome in liver transplant patients with HCV recurrence. We aimed at further verify our hypothesis through a multicenter randomized controlled study (clinicaltrial.gov: NCT00993122).
Methods: The study is a multicentre prospective randomized controlled trial comparing 8-weeks RBV treatment followed by 48 weeks of PegIFN-Riba (PR) therapy vs. 48 weeks of PR. Riba and PEG-IFN2b and were given according to the EASL guidelines. Randomization was stratified for HCV genotype. 4 Italian centers participate in patients enrollment according to the inclusion and exclusion criteria starting from 2011 for a consecutive period. Inclusion criteria were: post-transplant HCV recurrence observed at least 6 months after liver transplant. Patients were excluded if they failed to meet the standard eligibility criteria for PEG-IFN/RBV treatment. IL28B polymorphism was also examined.
Results: Forty-nine patients were randomized 1:1 to RBV pre-treatment (pre-treatment group) or PEG-IFN2b (standard group). Liver biopsy prior to therapy showed moderate fibrosis (F <2) in 54% of patients and severe fibrosis (F >3) in 46%. HCV genotype were 1b 78% and non-1 22%. During RBV pre-treatment, HCV RNA showed a decline of 0.64 log10 IU/mL compared to pre-treatment level ($P < 0.003$) that was unrelated to the IL28B rs12979860 genotype (CC vs CT/TT, $P = 0.320$). The rate of rapid virological response (RVR) was significantly higher in the RBV pre-treatment arm compared to the standard treatment arm ($P = 0.040$) while the SVR rate was similar (48% and 43%, respectively; $p > 0.300$). However, the risk to develop severe therapy-related side effects and the need for supportive therapy was higher in the standard compared to the pre-treatment arm (32 vs 18%, respectively; $P = 0.042$).
Conclusions: In conclusion, RBV pre-treatment showed a significant antiviral activity independent to fibrosis at baseline or IL28 genotype. Although RBV pre-treatment failed to achieve a better outcome (SVR), the reduced incidence of serious adverse events might favor this option.

P0065

EFFECT OF PORTAL VEIN THROMBOSIS (PVT) ON SURVIVAL AFTER LIVER TRANSPLANTATION (LT): A METANALYSIS

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Background and Aims: Portal vein thrombosis (PVT) is a common complication in patients with liver cirrhosis undergoing liver transplant (LT). Although PVT is no longer considered an absolute controindication to LT, published data of its effect on mortality after the surgery are heterogeneous and discordant. The aim of the present study was to systematically review the current literature on the role of PVT in LT recipients in term of outcome.

Methods: A systematic review of the English and non English literature was performed by analyzing studies that report on PVT in LT recipients and were published between January 1986 and January 2013. We performed a meta-analysis using the 30-day and 1-year mortality as endpoints in all the studies, using random effect model.
Results: Twenty-six studies among the total of 426 articles initially retrieved were considered. Of 25,753 LT, 2004 were performed in patients with PVT (7.78%), and approximately half had complete thrombosis (according to Yerdel's classification) at the time of LT. Seven studies report on 30-day mortality in both patients with PVT and without PVT, for a pooled mortality rate of 10.5% vs 7.7% (P = 0.01) respectively with OR equal to 2.29 (95%, CI 1.43–3.68). Nineteen studies report on 1-year mortality after LT in both patients with and without PVT, for a pooled mortality rate of 18.8% vs 15.3% (P = 0.001). The OR for 1 year mortality in patient with PVT was 1.39 (95%, CI 1.18–1.64). Four studies report on 30-day mortality in both patients with partial and complete PVT, for a pooled mortality rate of 5.9% vs 17.5% (P = 0.001) respectively, with OR equal to 5.65 (95%, CI 2.00–15.96). There wasn't heterogeneity between studies both for the 30-day and 1-year mortality.
Conclusions: Presence of PVT in liver transplant recipients increases 30-day and 1-year mortality, therefore screening and treatment of this complication in patient awaiting LT seems essential.

P0066
DIASTOLIC DYSFUNCTION IS PROGNOSTIC FOR MORTALITY IN LIVER TRANSPLANTED PATIENTS WITH NORMAL EJECTION FRACTION

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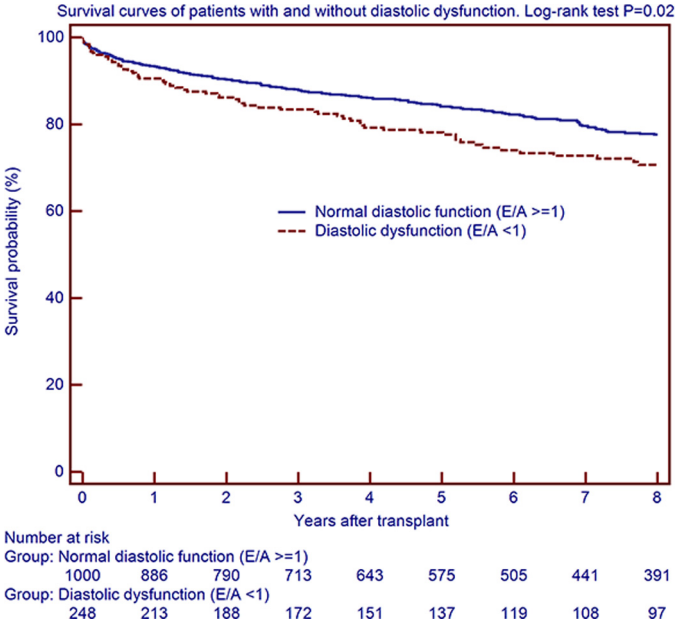
Background and Aims: Coronary artery disease and left ventricular ejection fraction (EF) are the main means of cardiovascular risk assessment of liver transplant (LT) candidates. The prognostic value of diastolic dysfunction, which is recognized as an early marker of cardiac risk in the general population, has not been studied in patients with cirrhosis. We aimed to evaluate the association between diastolic dysfunction in cirrhotic patients with normal EF and long-term mortality after LT.
Methods: Consecutive cirrhotic patients undergoing LT at a tertiary medical center between 2003 and 2013 were identified. Patients with combined heart and liver transplant, amyloidosis, hemochromatosis, sarcoidosis or carcinoid liver disease were excluded. 1,248 LT recipients with normal EF (>55%) were included. Diastolic dysfunction in cirrhosis was defined as a ratio of the early to late ventricular filling velocities (E/A ratio) value <1, according to the 2005 World Congress of Gastroenterology consensus. The association between baseline characteristics and estimated mortality was calculated using Cox Regression analysis.
Results: The prevalence of diastolic dysfunction was 20% in cirrhotic patients with EF ≥55%. Patients with diastolic dysfunction were older (59±7 vs. 51±11 years; p < 0.0001) and had greater preexisting cardiovascular comorbidities, diabetes, hypertension and hyperlipidemia compared to those with normal diastolic function (p < 0.05 for all). During median follow-up of 5.9 years after LT, the mortality of patients with diastolic dysfunction was 5.0% compared to 3.6% per person-year in those without diastolic dysfunction (p = 0.02) (Figure 1). Diastolic dysfunction was independently associated with an increase in all-cause mortality (adjusted hazard ratio 1.4, 95% confidence interval: 1.1–1.8; p = 0.02). Preexisting cardiovascular comorbidities, hypertension, hyperlipidemia and obesity were also independently associated with mortality.
Conclusions: Left ventricular diastolic dysfunction independently predicts post-LT mortality. This finding suggests that pre-transplant assessment of diastolic function adds an incremental value in predicting post-transplant mortality among cirrhotic patients with normal ejection fraction.



P0067
HEPATITIS C VIRUS INFECTION IS ASSOCIATED WITH LOWER SURVIVAL FOLLOWING LIVING DONOR LIVER TRANSPLANTATION IN THE U.S.

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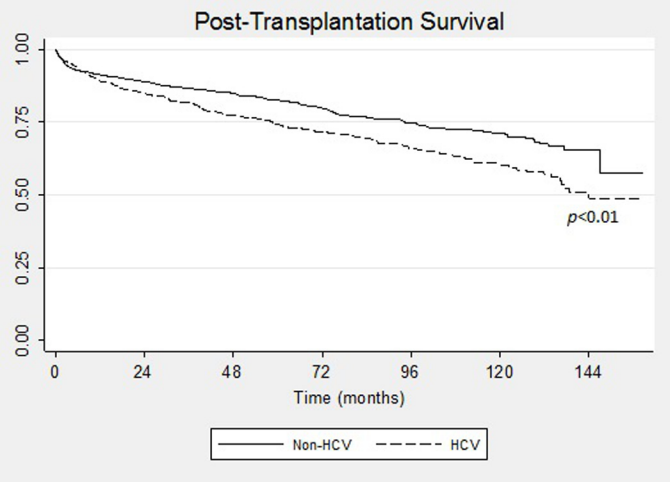
Background and Aims: Living donor liver transplantation (LDLT), well studied in Asia, may address the gap between the limited availability of deceased donor livers and the growing waiting list for liver transplantation. However, concern exists regarding potential increased long-term mortality following LDLT due to recurrent hepatitis C virus (HCV), which remains the leading cause of end-stage liver disease resulting in liver transplantation in the U.S. Nevertheless, large studies examining the association of HCV on long-term survival post-LDLT in the U.S. are lacking.
Methods: We conducted a retrospective cohort study using population-based national data from the United Network for Organ Sharing registry to evaluate the impact of HCV on long-term survival among adult patients undergoing LDLT in the U.S. from 1995 to 2012. Survival outcomes among patients

undergoing LDLT were stratified by HCV status. Post-LDLT survival was evaluated with multivariate Cox proportional hazards model adjusted for age, gender, race/ethnicity, obesity, hepatocellular carcinoma (HCC), Model for End-Stage Liver Disease score, ascites, hepatic encephalopathy, diabetes mellitus (DM), and year of liver transplantation.

Results: Overall, 2,258 adult patients underwent LDLT from 1995–2012, including 882 with HCV (39.1%) and 1,376 without HCV (60.9%). On multivariate Cox proportional hazards modeling, patients with HCV had significantly lower post-LDLT survival compared with non-HCV patients (HR 1.42, 95% CI, 1.10–1.83, $p < 0.01$) (Figure). HCC (HR 1.81, 95% CI, 1.25–2.63, $p < 0.01$), older age (HR 1.03, 95% CI, 1.02–1.04, $p < 0.001$), and DM (HR 1.52, 95% CI, 1.09–2.11, $p = 0.02$) were similarly associated with a significantly lower survival following LDLT. Patients with HE (HR 1.27, 95% CI, 0.97–1.65, $p = 0.08$) had a non-significant trend toward lower survival, whereas obesity (HR 1.08, 95% CI, 0.82–1.43, $p = 0.58$) was not associated with survival following LDLT.

Conclusions: Among U.S. adult patients undergoing LDLT for chronic liver disease, HCV infection is associated with significantly lower post-LDLT survival compared to non-HCV patients. HCC, older age, and DM are also associated with lower survival following LDLT. These findings may enable optimization of patient selection for LDLT and identification of a population that may benefit greatly from HCV eradication with recent availability of highly efficacious interferon-free antiviral therapy.



P0068

ALCOHOLIC ETIOLOGY AND INSULIN DEPENDENT DIABETES ARE ASSOCIATED TO THE PRESENCE OF CORONARY DISEASE IN CIRRHOTIC PATIENTS WITH BASAL CORONARY RISK

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Background and Aims: The prevalence of coronary artery disease (CAD) in patients who are been evaluated for liver transplantation (LT) seems to be higher than in the general population and related to a higher morbimortality after LT. However, there is no consensus for its diagnosis and treatment. Data about the potential risk factors are also scarce. Our objectives were (1) to analyze the prevalence of CAD diagnosed by angiography in a population of patients who were been evaluated for LT and had a high basal risk and (2) to determine the risk factors associated.

Methods: 203 patients were evaluated for LT in our centre (June 2012 to February 2014). The indications for the coronary evaluation (by direct angiography or CT-coronariography) were (1) symptoms or previous history of CAD; (2) imaging data of atherosclerosis in other localizations; (3) ≥3 cardiovascular risk factors (CVRF): male, >50 years, smoking, dyslipidemia and hypertension; (4) diabetes; (5) LVEF <50%. Stenosis >50% was defined as significant and revascularization was considered.

Results: The coronary study was indicated in 129 cases and it was eventually performed in 74 of them who did not have any other contraindication for LT (61 direct angiographies and 13 CT-coronariographies). The indications were: 43 cases (58.1%) with ≥3 CVRF, 26 (35.1%) for diabetes, 1 (1.4%) for atherosclerosis in other localizations and 1 (1.4%) for previous history of CAD. Three additional explorations were performed for other reasons. CAD of any severity was detected in 34 cases (45.9%). Eight of them (10.8%) had significant stenosis and 6 were treated. No adverse events were reported. After a median follow up period of 10.2 months, there were no coronary events in the 44 patients who received LT. Into the subpopulation of patients selected for the coronary evaluation, the group with CAD had more frequently alcoholic disease (70.6% vs 45.0%; p = 0.04), insulin dependent diabetes (IDDM) (29.4% vs 12.5%; p = 0.08), consumed a higher number of cigarettes per day (21.9 vs 13.3, p = 0.05) and had a higher MELD score (16.8 vs 12.8, p = 0.08). In the multivariate analysis, the alcoholism (OR 3.5 [95% CI 1.2–9.9]; p = 0.02) and IDDM (OR 3.8 [95% CI: 1.1–13.9]) were independent risk factors.

Conclusions: The prevalence of CAD detected by angiography in cirrhotic patients who are been evaluated for LT and have high basal risk is close to 50%. In this population, the alcoholism and IDDM are associated to a higher risk of CAD. The diagnosis and treatment of CAD in this population is safe.

P0069

RISK FACTORS FOR METABOLIC SYNDROME AFTER LIVER TRANSPLANTATION: WHAT HAS CHANGED OVER TIME

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Background and Aims: Post-Transplant Metabolic Syndrome (PTMS) is common among liver transplantation (OLT) recipients and may contribute to non-graft-related morbidity/mortality. The aim of this study is the definition of the factors contributing to PTMS development with particular regard to changes in the immunosuppressive regimens.

Methods: Three hundred and ninety-three patients who underwent OLT at Turin Liver Transplant Center were prospectively enrolled (group A: pre-mTOR inhibitors – OLT between 1998–2003, n = 167; group B: m-TOR inhibitors – OLT between 2008–2012, n = 226). Overall the median follow-up was 60 months (1–204), 144 (83–204) in group A and 48 months (1–60) in group B. Clinical, laboratory parameters, prescribed medications, clinical events/deaths were recorded every 6–12 months. Clinical features of the donors and histological data of the allograft were collected. Univariate and multivariate analysis were performed to identify risk factors associated with PTMS development.

Results: Clinical features and OLT indications were comparable between the two groups except for HBV and alcoholic cirrhosis as underlying liver disease (29.9% group A vs 20.9% group B, p = 0.033; 13.8% group A vs 18.2% group B, p = 0.004). In the whole cohort, cryptogenic cirrhosis was an OLT indication in 2.8% (11/393) without temporal changes. Overall pre-OLT MS was found in 1.8% (7/393) subjects, without temporal changes, while PTMS

in 24.9% (98/393; group A 37.9%, group B 22.6%, p = 0.003). Overall multivariate analysis identified pre-OLT hyperglycemia and post-OLT weight gain as predictive risk factors for PTMS. However, independent risk factors were different in the two groups: post-OLT weight gain (OR 1.53, 95% CI 1.26–1.85, p = 0.0001) and post-OLT US liver steatosis (OR 4.12, 95% CI 1.48–11.4, p = 0.006) in group A; post-OLT weight gain (OR 1.36, 95% CI 1.17–1.57, p = 0.0001), pre-OLT hyperglycemia (OR 3.57, 95% CI 1.28–9.88, p = 0.014), macrovacuolar steatosis ≥20% in the allograft (OR 4.74, 95% CI 1.23–18.27, p = 0.024) and treatment with m-TOR inhibitors (OR 17.43, 95% CI 3.36–90.39, p = 0.001) in group B. Cardiovascular (CVD) events were reported in 2.7% (10/393; group A 4.2%, group B 1.8%, p = ns). Overall 18.5% (73/393) died, 6.8% (5/73) for CVD (group A 2.1%, group B 0.9%, p = ns).
Conclusions: Overall, host-related factors contribute to PTMS development, but the introduction of mTOR inhibitors is an additional risk factor particularly when suboptimal livers are transplanted.

P0070
EFFICACY OF THERAPEUTIC ERCP IN BILIARY COMPLICATIONS FOLLOWING LIVER TRANSPLANT: 10-YEARS EXPERIENCE
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Background and Aims: Biliary tract complications following liver transplant remain an important source of morbidity and mortality. Endoscopic retrograde cholangiopancreatography (ERCP) has become a common therapeutic option before other invasive procedures. The aim of this study was to evaluate ERCP efficacy in managing these complications.
Methods: It was performed a retrospective study of all patients submitted to therapeutic ERCP due to post-liver transplant biliary complications, between September 2004 and September 2014, in a predominantly deceased donor liver transplantation center.
Results: Therapeutic ERCP was performed in 120 patients (64.2% men; mean age at first ERCP of 46.3±14.3years). Mean time between liver transplant and first ERCP was 19.8±38.8 months. Biliary complications were anastomotic strictures (AS) in 59.3%, non anastomotic strictures (NAS) in 14.1%, bile leaks in 5.9% and biliary filling defects (like stones or sludge) in 7.4%. Stents were placed in 61.7% patients, balloon dilation was performed in 62.5% and filling defects were removed in 29.5%. Each patient needed a mean of 3.27±2.40 ERCPs, performed during a period of 18.7±19.3 months. Mean follow-up was 32.9±30.5 months. Globally, biliary complications were successfully managed by ERCP in 43.3% of patients. Per complication, ERCP was successful in 41.2% of AS, 15.8% of NAS, 87.5% of leaks and 90.0% of lithiasis. One quarter of patients required a percutaneous or surgical intervention due to ERCP inefficacy. Diagnose of a bile leak (p = 0.001) or lithiasis (p = 0.003) was associated with higher chance of successful ERCP treatment, while the presence of a NAS was associated with unsuccessful ERCP (p = 0.001). No statistical difference was found for AS.
Conclusions: ERCP allowed resolution of a biliary complication in 43.3% of patients, avoiding a more invasive procedure. Endoscopic treatment is particularly efficient in patients with bile leaks, lithiasis and AS.

P0071
REAL-TIME MEASUREMENTS OF TISSUE OXYGEN MICROTENSION AS A MARKER OF BILE DUCT VIABILITY IN LIVER TRANSPLANTATION
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Background and Aims: The main aim was to evaluate bile duct viability by assessing its microvascular quality using an innovative real-time oxygen tension device by testing different areas in both donor and recipient's side. The findings were subsequently correlated with immunohistochemical and histopathological results. As a secondary aim, differences in bile duct micro-oxygenation status were analysed according to several donor, recipient and technical factors.
Methods: Observational prospective cohort study with 18 patients included from November 2013 to September 2014. Tissue oxygen microtension measurements were made using Oxylite® device in different areas of recipient and donor's bile duct intraoperatively after biliary anastomosis was made. Bile duct and hepatic artery biopsies were taken from donor and recipient.
Results: A total of 18 patients underwent liver transplantation with a median age of 53 years old (44–60) and MELD of 18 (17–26). Mean oxygen microtension value in the graft bile duct at anastomosis level was 106 (92–118) mmHg, being 125 (108–134) mmHg 1.5 cm proximal to the hilar plate. Mean micro-oxygenation value in the bile duct recipient was 117 (100–150) mmHg, whilst a value of 138 (119–183) mmHg was observed 1.5 cms distal to the anastomosis. Tissue oxygen microtension was statistically higher in distal areas to section border of the biliary anastomosis, with an overall pO2 increase distal to the anastomosis of 17.94 mmHg (p < 0.001) and 21.61 mmHg (p < 0.001) in the graft and recipient, respectively. Biliary anastomosis was performed above the cystic duct insertion in the donor bile duct in 10 patients, with significant higher values of pO2 microtension (p = 0.017). Histological injury grade 2–3 in biliary mural stroma and grade 1–3 in peribiliary vascular plexus of graft's bile duct graft were associated with lower tissue oxygen pressure, as well as injury grade 2 in biliary epithelium and grade 1–3 in peribiliary vascular plexus of recipient's bile duct were associated with lower micro-oxygenation (p < 0.05).
Conclusions: Biliary anastomosis is a critical point in liver transplantation. Our results demonstrates that terminal border of donor and recipient bile duct are low-vascularized areas. Tissue microoxygenation improves significantly in areas close to the hilar plate and to the duodenum in the donor and recipient's sides, respectively. Histopathological findings of bile duct injury are associated to worst tissue microoxigenation.

P0072
MICROINVASIVE INTRAOPERATIVE ULTRASOUND PATTERN IN PREDICTING OUTCOMES FOR SINGLE SMALL (<3 cm) HEPATOCELLULAR CARCINOMA
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Background and Aims: The significance of tumor microinvasion (portal venous, hepatic vein, or bile duct infiltration and/or intra-hepatic metastasis, MI) in patients with single, small (<3 cm) hepatocellular carcinoma (HCC) remains unclear. Aim of the study was to evaluate MI impact (MI^+ vs MI^-) on HCC recurrence and

long-term survival in patients treated by hepatic resection (HR) or surgical radiofrequency ablation (RFA).
Methods: All patients with single, small HCC who underwent HR or RFA between 1997 and 2013 were included in this study. In agreement with the histological criteria described by Yamashita, the intraoperative ultrasound (IOUS) definition of MI has been defined according to our previous report on IOUS HCC classification.
Results: 370 patients (141 HR) were included: 144 (39%) patients had MI^+: 33% in VE-HCC (<2 cm) and 49% in the others (2–3 cm) ($p = 0.002$). In the HR subgroup, IOUS findings were associated with histopathologic features ($p = 0.0001$), sensitivity to detect MI^+ being 84% and positive predictive value being 86%. MI+ patients had significantly worse survival than MI^- (5-year survival: 33% vs. 49%, respectively; $p = 0.0358$) and higher intra-hepatic recurrences (5-year HCC recurrences: 86% vs. 64%, respectively; $p = 0.0001$). No difference was found among MI^+ patients submitted to HR or RFA for long-term survival or intra-hepatic recurrence, while a higher rate of local recurrence was found after RFA (5-year local recurrences: 48% vs 9%, respectively; $p = 0.0001$).
Conclusions: In patients with single, small HCC, the prevalence of MI^+ is high, even in cases of HCC <2 cm. IOUS findings strongly correlated with histopathologic criteria in detecting MI^+. MI^+ HCC patients had a worse prognosis, irrespective to treatment. IOUS MI pattern (during a bridging treatment) may represent a valid tool in the selection of HCC patients for transplantation.

P0073
INDIVIDUALIZING HBIg REINFECTION PROPHYLAXIS AFTER HBV-INDUCED LIVER TRANSPLANTATION: RESULTS FROM A MATHEMATICAL MODELING APPROACH BASED ON CLINICAL DATA

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Background and Aims: Although hepatitis B immune globuline (HBIg) administration in combination with hepatitis B virus (HBV) polymerase inhibitors is the standard of care for prophylaxis of reinfection after liver transplantation (LTX) for HBV-related liver disease in many transplant centers worldwide, no rational basis for HBIg doses schedules exists until today. Individualized treatment regimens are of particular interest for high risk patients, HDV-coinfected patients (virtually no treatment options in case of reinfection), and because of the high costs and limited availability of HBIg.
The objective of the present study is the mathematical analysis of the virus kinetics after HBV-induced LTX and pharmacokinetics of HBIg after LTX.
Methods: Serial quantifications of anti-HBs and HBsAg for 67 patients were available for this retrospective analysis. All patients were transplanted at Hannover Medical School between 1994 and 2012. For part of these patients, clinical data was already published (Rosenau et al., 2007, Mederacke et al, 2012). We developed and evaluated new virus kinetics models basing on existing approaches (Nowak et al., 1996, Mager and Jusko, 2001, and Gourley et al., 2008). A potential reinfection of the graft was considered as well as HDV-coinfection during the modeling process. Model parameters were estimated by non-linear fitting of individual patient data.
Furthermore, a simulation study was performed to evaluate potential therapeutic regimes based on individual parameters. The defined goal of treatment in this simulation study was to decrease HBsAg under the detection limit within 11 days after LTX. Secondary criterion was the cost-effectiveness of the analyzed therapeutic regimes.
Results: In our simulations, we estimated that patients with a serum HBsAg level over 5,000 IU at the time of LTX benefited most likely from a therapy regime with a single dose of 20,000 IU during the anhepatic phase and subsequently 10,000 IU daily, while patients with a HBsAg-level below 5,000 IU profited from a therapy regime with a single dose of 10,000 IU and 4,000 IU subsequently. Patients with serum-HBsAg below 100 IU needed a single dose of 10,000 IU only. Overall, simulation results suggest treatment individualization according to baseline HBsAg-levels as a reasonable and feasible approach.
Conclusions: We propose an approach to model pharmacokinetics and pharmacodynamics of HBIg after HBV-induced LTX. The modeling results reveal potential risk factors and form a basis for treatment individualization.

P0074
PREVALENCE AND RISK FACTORS OF METABOLIC SYNDROME AFTER LIVER TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

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Background and Aims: Metabolic syndrome (MS) affects more than half of liver transplant (LT) recipients, increasing long term mortality and morbidity. However, few studies have evaluated the short and long-term prevalence of the MS post-LT and its potential risk factors. The aim of this study was to evaluate the short and long-term prevalence and the potential risk factors of MS after LT.
Methods: Patients who underwent LT at the Padova Liver Transplant Center between January 2000 and March 2013 (retrospective cohort) and between April 2013 and April 2014 (prospective cohort) and who were followed-up at Multivisceral Transplant Unit were included. Patients <18 years, who underwent multiorgan transplantation or re-LT and with MS diagnosed at the time of LT were excluded. For each patient general and metabolic variables, donor characteristics, transplant variables were recorded.
Results: In the retrospective cohort 161 patients (120 M, 41 F) were included. Mean±SD age at transplant was 52.5±9.5 years. The most common indication to LT was HCV-related cirrhosis (49.1%). A post-LT significant increase in BMI values, in diabetes and hypertension prevalence and in total cholesterol and triglyceride levels was found compared to pre-LT values. At a mean post-LT follow-up of 6.9±4.2 years 81/161 (50.3%) patients developed MS. Recipient male sex (OR 2.36, $p = 0.045$), a higher pre-LT BMI (OR per unit 1.14, $p = 0.03$), and the presence of pre-LT diabetes (OR 5.98, $p = 0.04$) were associated with the development of post-LT MS. In the prospective cohort 15 patients were included (10 M), mean±SD age at LT of 52.2±5.8 years. HCV-related cirrhosis was the most common indication to LT (33%). At 3, 6 and 12 months after LT a significant increase in BMI values, diabetes and hypertension prevalence and in cholesterol and triglyceride levels was found compared to pre-transplant values and 5/15 (33.3%), 3/11 (27.3%) e 4/10 (40%) patients developed MS.
Conclusions: Post-LT MS affects nearly half of LT recipients, starting early after LT. Recipient male gender, pre-LT diabetes and increased BMI are risk factors for MS after LT. Lifestyle modifications, should be recommended to transplanted recipients, especially in the overweight/obese ones, starting in the early post-LT period. This would facilitate prevention of body weight gain and the associated abnormalities, thus reducing incidence of post-LT MS and the related cardio-vascular events.

P0075

HEALTH-RELATED QUALITY OF LIFE IS IMPROVED AFTER LIVER TRANSPLANTATION AND IS RELATED TO DISEASE ACCEPTANCE, HELPLESSNESS AND PERCEIVED DISEASE BENEFITS

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Background and Aims: Liver transplantation (LT) is the only curative treatment for end-stage liver disease (ESLD) with excellent long-term outcomes, regarding morbidity and mortality rates. However, an important outcome parameter is health-related quality of life (QoL). Improvement of QoL has been described after liver transplantation. We wanted to confirm these findings with specific attention for psychological parameters such as acceptance, helplessness and perceived disease benefits.
Methods: We performed a cross-sectional study (pre-post design) in a liver transplant unit. Self-report questionnaires were conducted in 120 patients with ESLD: 60 patients pre-transplantation and 60 post-transplantation. A control group of 57 patients with ESLD without the perspective of a transplantation also completed the same questionnaires on QoL (SF-36), acceptance (ICQ), helplessness (ICQ) and disease benefits (ICQ). Clinical and socio-demographical data were collected from the medical files. Data were analysed using the Mann–Whitney U test and Spearman's rank correlation coefficient.
Results: The studied groups of patients were comparable regarding age and MELD score, but the control group was significantly older ($p = 0.09$). We observed a significant increase in QoL as soon as 3 months after LT ($p = 0.046$) as well regarding the mental component summary scale ($p = 0.029$) as the physical component summary scale ($p = 0.033$). After liver transplantation, patients report more acceptance ($p < 0.01$) and disease benefits ($p < 0.0001$) and a decrease in helplessness ($p < 0.05$). General QoL is positively significant correlated ($p < 0.001$) with acceptance ($r_s = 0.737$) and disease benefits ($r_s = 0.494$), and negatively with helplessness ($r_s = -0.828$).
Conclusions: These data confirm an increase of QoL starting from 3 months after liver transplantation. We observed a better acceptance of their illness and more benefits of the illness after transplantation. Patients also report to feel less helpless, but the decrease is less distinct. We also found a high association between the QoL and helplessness. We assume that these findings indicate that patients receive a lot of attention and support for their illness after transplantation but still feel slightly uncertain and helpless about the future, regarding coping with it (medication, diet, …). These aspects deserve more attention and could give new directions in the approach of liver patients after transplantation. Further research on this matter is necessary.

P0076

ALCOHOL RELAPSE AND CARBOHYDRATE DEFICIENT TRANSFERRIN MEASUREMENT AFTER LIVER TRANSPLANTATION FOR ALCOHOLIC LIVER CIRRHOSIS

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Background and Aims: End-stage alcoholic liver disease (ALD) is the second most-common indication for LT in Europe. However, it induces more debate than any other indication, mainly due to apprehensions about the abstinence period before listing to LT. Long-term observation after LT is challenging and biomarkers e.g. carbohydrate deficient transferrin (CDT) may help identifying a possible alcohol relapse. We intended to analyse the outcome of patients with LT due to ALD at the Medical University of Vienna.
Methods: A retrospective analysis of prospectively collected data from patients listed for LT with ALD as main or secondary indication between 1996 and 2012 was performed. Primary endpoints were long-term patient and graft survival as well as incidence of alcohol relapse. The latter was strictly defined as any post-LT alcohol consumption, evaluated by a specialist psychologist. A defined period of sobriety before LT was not required at our institution. CDT-levels were measured regularly after LT and were correlated with clinical outcome.
Results: From 1996 to 2012, 458 patients were listed for LT due to ALD as main or secondary indication. Out of these, 382 (83%) were transplanted, 36 (8%) died on the waiting list and 40 (9%) were removed from the waiting list. From patients who received LT, ALD was the main indication in 290 and the secondary indication in 90 patients. The median follow-up amounted 73 months (0–213). One-, and 5-year patient and graft survival was 82%, 69% and 82%, 75%, respectively. The alcohol relapse rate at 1- and 3-years showed 4.8% and 12.9%, respectively. Patients with ALD as main indication experienced significantly more often an alcohol relapse (log rank; $p = 0.037$). CDT levels were measured regularly post-LT and showed a sensitivity of 94% and a specificity of 87%. 186 of 382 patients died during the observation period, 32 with an alcohol relapse and 154 without. In patients who died with alcohol relapse, death was significantly more often liver-related than in patients without alcohol relapse (Chi-squared test; $p < 0.0001$). Alcohol relapse and ALD as main or secondary indication for LT did not significantly affect long-term patient and graft survival.
Conclusions: This large single centre analysis presents excellent long-term outcome in patients with LT for ALD. Our alcohol relapse rate was low compared to published data, although a strict definition was applied. Furthermore, post-LT CDT-measurement proofed to have high sensitivity for diagnosing alcohol relapse.

P0077

IMPROVEMENT OF LIVER ELASTICITY IN HCV-PATIENTS UNDER SOFOSBUVIR-BASED THERAPY AFTER LIVER TRANSPLANTATION

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Background and Aims: Recurrence of HCV and its related progression of fibrosis after liver transplantation (OLT) are well documented. So far, interferon (IFN) based antiviral therapy represents the hallmark in treating HCV patients also after OLT. Neither sustained virologic response (SVR) nor safety profiles of the INF-based therapies are satisfactory for transplant recipients. In January 2014 the direct-acting antiviral (DAA) drug sofosbuvir (SOF) was introduced in Germany followed by simeprevir in May 2014 and daclatasvir in August 2014. Efficacy and safety of these newly introduced drugs are promising, experience in interactions are limited. The purpose of this ongoing analysis is to circumvent the progression of fibrosis in HCV liver transplant recipients.
Methods: Patients post OLT presenting with biopsy proven HCV inflammation were/are treated with SOF and Ribavirin (RBV) for 24 weeks ($n = 13$) or SOF in combination with another DAA ($n = 3$) for 12 weeks. Laboratory parameters and Fibroscan® were/are assessed constantly every fourth week during the treatment and 12 weeks after the end of the regimen. As control group HCV liver transplant recipients who received originally antiviral therapy with pegylated INF and/or RBV and/or Telaprevir (TLV) were retrospectively evaluated.

Results: Nine out 13 subjects with SOF/RBV had a rapid virologic response (RVR) and additional 4 patients developed an early virologic response (EVR). Comparing the two studied groups the rate of RVRs is significantly higher in the SOF-based therapies (p = 0.025, Fisher's exact test, 2-sided). All patients whose treatment ended at least 12 weeks ago show a SVR (n = 5). The three patients treated with two DAAs developed a RVR. Four patients who experienced a failure of an INF-based antiviral response responded to SOF/RBV. Preliminary data on Fibroscan® analysis revealed 12 patients with regression of fibrosis during the treatment with SOF in combination with RBV or another DAA. There were no side effects observed, which resulted in premature termination of SOF.
Conclusions: SOF seems to be a safe and effective therapeutic option in OLT patients infected with HCV. Preliminary results show that regression of fibrosis after OLT is feasible within 24 or 12 weeks of therapy at least in a subgroup of patients. This new interferon-free era of treating HCV will likely result in improved outcome of both patient and transplant survival in transplant recipients being infected with HCV.

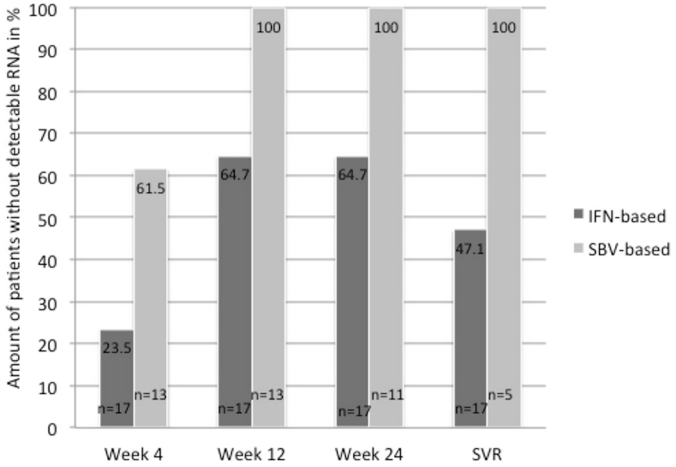

Figure: Progress of virologic response. The percentages of patients with no detectable HCV-RNA at different stages of the therapy with SOF and RBV are illustrated. The absence of detectable RNA in week 4 is defined as a rapid virologic response (RVR), in week 12 as an early response (EVR) and 24 weeks after the end of the therapy as a sustained response (SVR). The higher rate of RVRs under SBV is significant (p = 0.025, Fisher's exact test, 2-sided) and very promising, as a fast response was an important factor indicating a future success of the therapy in several other studies concerning HCV.

P0078
LAPAROSCOPIC RADIOFREQUENCY ABLATION VERSUS HEPATIC RESECTION IN THE TREATMENT OF VERY EARLY HEPATOCELLULAR CARCINOMA IN CIRRHOTIC CARCINOMA: A COHORT STUDY

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Background and Aims: Available studies showed conflicting results concerning survival rates for patients with cirrhosis and concurrent very early (VE) hepatocellular carcinoma (HCC) (stage 0 according BCLC classification: single <2 cm or carcinoma in situ, Child A) treated by laparoscopic radiofrequency ablation (LRFA) or hepatic resection (HR). Even if very few comparative studies between HR and RFA have been reported for VE-HCC, some experts have recommended modification of BCLC therapeutic algorithm in this subgroup of patients: RFA should be the first-line therapy and HR should be considered only in patients with failure of or contraindications to RFA. We compared HR versus LRFA as initial treatment for VE-HCC on survival, disease free survival (DFS) and HCC recurrences in a large cohort of patients followed in clinical practice
Methods: Two independent cohort series (Milan, Creteil), which include 855 patients with HCC who underwent HR (n = 529) or LRFA (n = 326), were retrospectively analysed. 176 patients had a VE HCC. Patients not suitable to HR (requiring a very large parenchymal loss at HR, due to the position of the lesion) were treated by LRFA (n = 92), the others underwent HR (n = 84). At the time of surgical procedure no relevant differences were seen between the 2 groups in term of liver disease status. In any case, Kaplan–Meier method was used to assess overall survival (OS), tumour recurrence rate and disease-free survival (DFS) before and after propensity score matching
Results: The 1-, 3- and 5-year OS rates for the LRFA group and the HR group were 92%, 66%, 46% and 94%, 89%, 75% respectively. The corresponding DFS rates for the 2 groups were 64%, 30%, 14%, and 82%, 58%, 39%, respectively. OS and DFS were significantly lower in the RFA group than in the HR group (p = 0.0029 and p = 0.0002, respectively). The 5-year HCC recurrence rate was higher in the LRFA group than in the HR group (82% vs. 56%; p = 0.0008). After propensity score matching, only survival rates become not significantly different. Multivariate analysis confirmed that treatment and BCLC stage are the only predictive factors for survival and intra-hepatic recurrences
Conclusions: HR yielded better disease-free survival rates than LRFA with not significant difference in survival. HR should be preferred in patients with peripheral tumours or with tumours located near gallbladder, main biliary ducts, bowel loops or big vessels, in which RFA may be less effective, if not dangerous. HR is the first-line option treatment for patients with VE-HCC

P0079
NUCLEOS(T)IDE ANALOGUE PROPHYLAXIS AFTER HEPATITIS B IMMUNOGLOBULIN WITHDRAWAL IS SAFE AND EFFECTIVE AGAINST HEPATITIS B AND D RECURRENCE AFTER LIVER TRANSPLANTATION

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Background and Aims: nucleos(t)ide agents (NAs) have made hepatitis B immunoglobulin (HBIG)-sparing protocol an attractive approach against hepatitis B virus (HBV) recurrence after liver transplantation (LT). However, this approach is considered controversial in patients transplanted for HBV and hepatitis D (HDV) coinfection.
Methods: all patients transplanted for HDV/HBV cirrhosis (7/1997 to 3/2013) were prospectively evaluated. After LT, each patient received HBIG (1,000–10,000 IU IV/IM in anhepatic phase and daily for 7 days and then monthly 1,000–2,000 IU IM) for a median time of 18 (range: 6–144) months plus NA and then continued with

NA mono- or dual prophylaxis. All patients were followed up with HBV serum markers and HBV-DNA, while anti-HDV/HDV-RNA was performed in those with HBV recurrence

Results: 34 recipients (all but 1 with undetectable HBV-DNA at LT; all with detectable HDV-RNA at LT) were included (11 men, mean age: 46.7±16 years). After HBIG discontinuation, NA(s) was received as monoprophylaxis (lamivudine: 2, adefovir: 1, entecavir: 9, TDF: 12) or as dual NA prophylaxis [lamivudine+adefovir (or tenofovir) in 10 patients]. None of the patients had HBV/HDV recurrence during combination of HBIG/NA(s) administration. However, two (5.8%) of the 34 patients had HBV/HDV recurrence after HBIG withdrawal [median follow up period: 28 (range 12–58) months]. These 2 patients had undetectable HBV-DNA but positive HDV-RNA at LT and they were under combination of lamivudine+adefovir prophylaxis at the time of recurrence. Statistical analysis revealed no factor significantly associated with HBV/HDV recurrence (pre-LT characteristics, antiviral protocol and immunosuppressive regimen post-LT), but those with recurrence had received HBIG for shorter period, compared to those without recurrence (median: 9 vs 20 months, p = 0.008). Glomerular filtration rate was not different between nucleoside and nucleotide (±nucleoside) analogue groups and similar regarding their serum calcium and serum phosphorus.

Conclusions: we showed for the first time that maintenance therapy with NA(s) prophylaxis after HBIG discontinuation was effective against HBV/HDV recurrence, but it seems that longer period of HBIG administration is needed before its withdrawal after LT.

P0080

HIGHER SURVIVAL IN BABY BOOMERS FOLLOWING LIVER TRANSPLANTATION: AN ANALYSIS OF THE UNOS DATABASE

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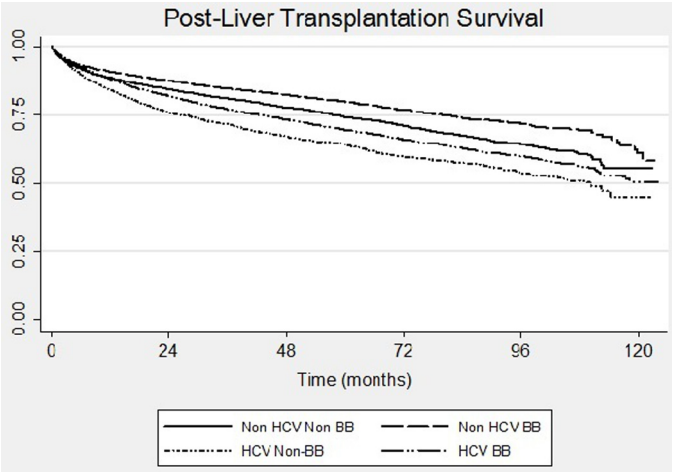
Background and Aims: According to the Centers for Disease Control and Prevention, individuals born between 1945 and 1965, the "baby boomer (BB) cohort", accounted for 81% of all chronic hepatitis C virus (HCV)-infected patients in the U.S. A significant proportion of HCV-infected BB have already developed complications of HCV-related cirrhosis, including hepatocellular carcinoma (HCC) and hepatic decompensation requiring liver transplantation (LT). The impact of the emerging BB generation on survival following LT in the U.S. remains unclear.

Aim: To evaluate the impact of birth cohort and HCV status on survival following LT among U.S. adult LT recipients.

Methods: Utilizing population-based national data from the United Network for Organ Sharing (UNOS) registry from 2003 to 2012, we evaluated birth cohort-specific (BB vs. non-BB) and etiology-specific (HCV vs. non-HCV) patient survival following LT among U.S. adults. Overall survival following LT, stratified by birth cohort and HCV status, was analyzed with Kaplan–Meier methods and multivariate Cox proportional hazards models adjusted for age, sex, HCC, body mass index, race/ethnicity, year of LT, diabetes mellitus, Model for End-Stage Liver Disease (MELD) score, albumin, ascites, hepatic encephalopathy, and cardiac disease.

Results: From 1995 to 2012, there were 89,479 LT performed in the U.S.: 68.0% (n = 60,842) among BB and 32.0% (n = 28,637) among non-BB. During the MELD era, from 2003 to 2012, overall patient survival following LT was the highest among the non-HCV BB cohort and the lowest among the HCV non-BB cohort. Among patients with HCV, BB patients had significantly higher 5-year survival following LT compared to non-BB patients (69.5% vs. 64.2%, p < 0.001) [Figure]. Among patients with non-HCV-related liver disease, similar trends were seen, with significantly higher 5-year survival following LT among the BB cohort compared with the non-BB cohort (79.8% vs. 74.2%, p < 0.001) [Figure]. Based on multivariate Cox proportional hazards models, compared to non-BB patients, patients in the BB cohort had significant higher post-LT survival (HR, 0.87; 95% CI, 0.81–0.93, p < 0.001).

Conclusions: BB patients demonstrated a significantly higher survival following LT compared to non-BB cohort patients. While survival following LT among HCV patients in our current study confirms previously reported outcomes, we also report a novel and significant birth cohort effect on post-LT survival. Further studies are needed to understand the survival advantage in the BB cohort.



P0081

REDUCED CALCINEURIN INHIBITOR EXPOSURE EARLY AFTER LIVER TRANSPLANTATION PROTECTS AGAINST RENAL DYSFUNCTION INDEPENDENTLY OF THE USE OF BASILIXIMAB INDUCTION THERAPY

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Background and Aims: Basiliximab use with delayed entry of tacrolimus (TAC) as renal sparing agent may help reduce renal impairment in liver transplant (LT) recipients. We aimed to evaluate factors affecting renal dysfunction and the effect of basiliximab post-LT.

Methods: All consecutive LT patients (1/2006–1/2014) with a follow up >3 months and eGFR <90 ml/min/1.73m^2 at day 1 post-LT were analyzed for factors known to affect renal dysfunction: biochemical/clinical data, donor age, cold/warm ischemia time, mean trough TAC levels up to days 7, 15, 30 post-LT, basiliximab use, initial/maintenance immunosuppression, acute cellular rejection (ACR), diabetes mellitus (DM) pre and post-LT, systemic hypertension, days on filtration post-LT. Renal impairment was defined as mild: eGFR 60–89and moderate/severe: eGFR <60. Endpoints were eGFR at 3 months and at last follow up post-LT.

Results: 126 patients given basiliximab were compared with 189 consecutive patients with eGFR <90 at day 1 post-LT (control group). Basiliximab group had mean TAC trough levels 3.4 ng/ml vs 5.9 ng/ml of controls at the first week and 4.2 ng/ml vs

6.6 ng/ml respectively at 15 days post-LT. 20 (16%) on basiliximab had ACR vs 72/188 (38%) controls (p = 0.001). Of those not on basiliximab, 35% (35/97) with TAC levels >5 ng/ml vs 41% (38/92) with TAC <5 ng/ml at week 1 post-LT experienced ACR episodes (p = NS).
Logistic regression analysis showed that factors associated with moderate–severe renal impairment (eGFR <60) at 3 months post-LT were: recipient age >50 years (p < 0.001, OR = 1.06, 95% CI 1.03–1.1), mean CNI levels >5 ng/ml at week 1 post-LT (p = 0.003, OR = 0.4, 95% CI 0.3–0.7) and mean CNI levels >7 ng/ml up to 15 days post-LT (p = 0.019, OR = 0.85, 95% CI 0.75–0.97). The same factors were associated with mild renal impairment (eGFR <90).
Factors associated with moderate/severe renal impairment at last follow up (median 48 mo) in the Cox regression analysis were: mean TAC levels >7 ng/ml at day 15 post-LT (p = 0.003, OR = 1.9, 95% CI 1.2–2.9) and use of steroids >3 months post-LT (p = 0.006, OR = 0.7, 95% CI 0.5–0.9).
Conclusions: Basiliximab use allows reduced TAC trough levels with less episodes of ACR compared with the control group. However, TAC trough levels <7 ng/ml at 15 days post-LT regardless of basiliximab use, were protective of renal function without predisposing to ACR in patients with renal impairment at baseline.

Liver transplantation/Surgery: c. Acute liver failure – clinical & experimental

P1294
PROOF-OF-PRINCIPLE EVALUATION OF IMMUNOMODULATORY DRUGS IN PROMOTING PHAGOCYTOSIS CAPACITY IN PATIENTS WITH LIVER FAILURE

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Background and Aims: Secondary infections are the main cause of death in patients with acute- (ALF) and acute-on-chronic liver failure (ACLF) and have been attributed to monocyte dysfunction. The use of steroids in these patients is common but controversial. Beneficial effects on haemodynamics do not confer survival benefit. Their immunomodulatory effect in liver failure syndromes is poorly understood. Steroid clearance was found decreased in critically ill patients. We determined monocyte phagocytosis in patients with liver failure and developed an ex-vivo proof-of-principle evaluation of drugs aimed at boosting innate immune responses.
Methods: Phagocytosis of *E. coli* and oxidative burst by monocytes was studied ex-vivo on monocytes from patients with ALF (n = 24), ACLF (n = 26), cirrhosis (n = 5) and compared to healthy controls (HC, n = 16) using pHrodo assay (Invitrogen) and Phagoburst (Glycotope). Immunophenotype (CD14, CD16, HLA-DR, CD163, MERTK, Fcy-receptors CD32, CD64) was assessed by flow cytometry. PBMCs from ALF/ACLF patients were treated ex-vivo with Interferon-γ (IFN-γ, 50 ng/ml), N-acetylcysteine (NAC, 250 mg/l) and hydrocortisone (500–10,000 nmol/l) for 48 h. Baseline plasma cortisol levels were assessed by liquid chromatography-mass spectrometry.
Results: Phagocytosis of *E. coli* was impaired in patients with ALF compared to HC (p < 0.0001) and ACLF compared to cirrhosis and HC (p = 0.004/p = 0.0028); and correlated negatively with activation marker CD163 (r = −0.365, p = 0.043). Oxidative burst was impaired in ALF (p = 0.0369) but not ACLF (p = 0.1275/p = 0.9897). Expression of Fcy-receptors was preserved. Ex-vivo treatment with IFN-γ (p = 0.0133) and NAC (p = 0.0156) decreased, and hydrocortisone at doses up to 2000 nmol/l increased phagocytosis (500 nmol/l, p = 0.0039; 1000 nmol/l, p = 0.0137; 2000 nmol/l, p = 0.371). Plasma cortisol levels >2000 nmol/l in patients on hydrocortisone treatment were associated with impaired phagocytosis (p = 0.0401) and high 28-day mortality (83.3%).
Conclusions: Defective phagocytosis occurs in patients with ALF and ACLF and may impair host defence. IFN-γ and NAC inhibited, while hydrocortisone enhanced monocyte phagocytosis capacity ex-vivo. High plasma cortisol levels in patients on hydrocortisone treatment conferred phagocytic defect and poor outcome. Decisions to use approved agents with immunomodulatory effects in patients with liver failure syndromes need careful consideration, hydrocortisone dosing and drug-monitoring require evaluation in clinical studies.

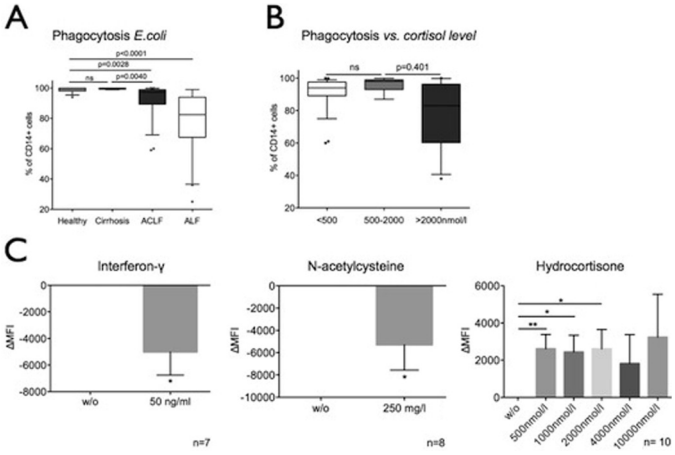

Figure: (A) Ex-vivo phagocytosis of *E. coli* in patients with ACLF and ALF compared to healthy controls and stable cirrhotic patents. (B) Ex-vivo phagocytosis in ACLF in relation to the baseline plasma cortisol level. (C) Phagocytosis after ex-vivo treatment of rnonocytes from patients with liver failure with interferon-γ, N-acetylcysteine and hydrocortisone for 48 h.

P1295
ROLE OF CK 18 (M30) IN PREDICTING MORTALITY IN PATIENTS WITH ACUTE LIVER FAILURE

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Background and Aims: Poor prognosis of acute liver failure (ALF) is well known. Although numerous prognostic criteria's have been established, they lag in their predictive accuracy when applied to Asian population. Also with the recent interest in cell death markers, the study was undertaken to assess the role of Cytokeratin 18 (M30) in ascertaining the prognosis of patients with ALF and thus identify who all will benefit from a liver transplant.
Methods: Forty-five adult patients with ALF were evaluated and managed in ICU in the Department of Medicine, of Lok Nayak hospital, New Delhi, India. Besides the liver function test and serological markers for HbsAg, IgM anti HCV, IgM anti-HAV, IgM anti-HEV and CK 18 (M30) levels were done by ELISA at base line and on follow up course in the hospital.
Results: The mean cleaved CK18 [M30] levels in ALF patients who survived were 767.37±247.97mIU/ml and in those who expired

were 1094.67±239.37 mIU/ml. The difference in values between these groups was statistically significant (p = 0.004).
There was a statistically significant difference in total leucocyte count, serum creatinine, INR and cleaved CK18 M30 levels among those that survived vs expired. Multivariate analysis found that all these independently predict mortality. A receiver operating characteristic curve was drawn for the new score. The score found was compared with the King's College criteria and the MELD score, the new scoring system was found to have higher sensitivity and specificity for mortality compared with other standard prognostic markers in ALF, and has a better diagnostic accuracy (Table 1).

Table 1. Sensitivity and specificity for mortality

	King's College Hospital Criteria	MELD score (≥20.96)	New score (≥0.15)
Sensitivity	69.4%	91.7%	94.4%
Specificity	88.8%	88.9%	89.9%
Positive predictive value	96.1%	96.9%	94.4%
Negative predictive value	42.10%	66.7%	77.7%
Diagnostic accuracy	73.3%	88.9%	91.1%
AUROC	–	0.944	0.978

The predicted probability of mortality can be found from the equation:
COMPUTED SCORE = −0.404 + (0.074·INR) + (0.00004513·TLC) + (0.0001·M30).
Conclusions: The results of our study suggest that initial serum CK 18 (M30) levels predict outcome in ALF patients. The ALF patients with serum Cleaved CK 18[M30] levels at admission more than 988.07 mIU/ml are candidates for liver transplantation, which can be life saving for these patients.

P1296
BLOCKING NMDA RECEPTORS PROLONGS SURVIVAL IN RATS WITH ACUTE LIVER FAILURE BY DUAL PROTECTIVE MECHANISMS IN KIDNEY AND BRAIN

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Background and Aims: Treatment of patients with acute liver failure (ALF) is unsatisfactory and mortality remains unacceptably high. Blocking NMDA receptors delays or prevents death of rats with ALF. The underlying mechanisms remain unclear. Clarifying these mechanisms will help to design more efficient treatments to increase patient's survival. The aim of this work was to shed light on the mechanisms by which blocking NMDA receptors delays rat's death in ALF.
Methods: ALF was induced by galactosamine injection. NMDA receptors were blocked by continuous MK-801administration. Edema and cerebral blood flow were assessed by magnetic resonance. The time course of ammonia levels in brain, muscle, blood and urine; of glutamine, lactate and water content in brain, glomerular filtration rate and kidney damage and of hepatic encephalopathy (HE) and intracranial pressure were assessed.
Results: ALF reduces kidney glomerular filtration rate (GFR) as reflected by reduced inuline clearance. GFR reduction is due to both reduced renal perfusion and kidney tubular damage as reflected by increased Kim-1 in urine and histological analysis. Blocking NMDA receptors delays kidney damage, allowing transient increased GFR and ammonia elimination which delays hyperammonemia and associated changes in brian. Blocking NMDA receptors does not prevent cerebral edema or blood–brain barrier permeability but reduces or prevents changes in cerebral blood flow and brain lactate.
Conclusions: Dual protective effects of MK-801 in kidney and brain delay cerebral alterations, hepatic encephalopathy, intracranial pressure increase and death. NMDA receptors antagonists may increase survival of patients with ALF by providing additional time for liver transplantation or regeneration.

P1297
DO MOLECULAR ALTERATIONS IN THE HEV GENOME PLAY A KEY ROLE IN ACUTE LIVER FAILURE?

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Background and Aims: Hepatitis E virus is a key public health disease in many developing countries and is also endemic in some industrialized countries. Very few studies have reported about the nucleotide substitutions in HEV and its association with acute liver failure (ALF). The present study was designed to assess the substitutional profile in the HEV isolates with acute liver failure and acute viral hepatitis from North Indian population. Besides, it was also aimed to associate the substitutions with the poor outcome of the disease.
Methods: A total of 42 patients with acute liver failure and 205 patients with acute viral hepatitis were screened for the study during the years 2011–2014. HEV IgM was detected by anti HEV IgM ELISA. HEV RNA was detected using Viral RNA extraction kit. The RNA Dependent RNA polymersase (RdRp) region of the Open Reading Frame 1 (ORF 1) protein of the HEV genome was amplified using Reverse Transcriptase PCR. HEV viral load was detected using Real time PCR. Representative samples were directly sequenced. Full length nucleotide sequences of HEV isolates were retrieved from the Gen Bank /EMBL/DDBJ databases and compared with the reference strains. Sequences were aligned by CLUSTAL W software.
Results: The mean age of the AVH and ALF patients were 23.82±2.88 years and 24.7±3.42 years respectively. HEV RNA was detected in 105 (51.21%) AVH and 31 (73.8%) ALF patients respectively. The substitutions 4476C>G and 4616A>C were present in 45.45% (25/55) of all the sequences amplified for the ORF 1 region while they were present in 100% (25/25) of the patients with acute liver failure. The substitutions were also found to be significantly different (p < 0.0001) when compared to patients with AVH. At the amino acid level, the Cysteine1483Tryptophan and Asparagine 1530 Threonine mutations were the most significant mutations (p < 0.0001) obtained in 100% (25/25) of the patients with acute liver failure compared to none (0/30) of the patients with AVH and the viral load corresponding to these 25 samples was significantly higher 58627.56±47029.83 copies/ml than 5682.16±4982.74 copies/ml (p < 0.0001) of the other isolates lacking these mutations.
Conclusions: The nucleotide substitutions in the RdRp region may play a crucial role in enhancing HEV replication thus leading to disease severity. The amino acid substitutions had a significant association with the poor outcome in ALF patients.

P1298

HYPOXIC PRECONDITIONING POTENTIATES TROPHIC EFFECTS OF MESENCHYMAL STEM CELLS ON CO-CULTURED HUMAN PRIMARY HEPATOCYTES

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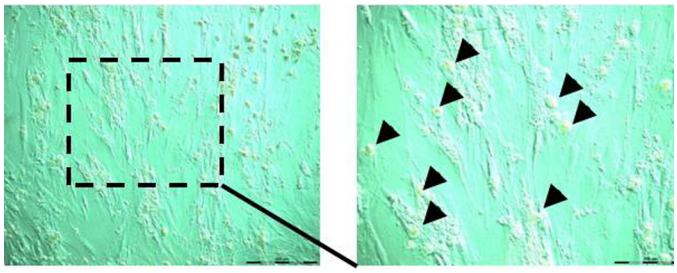
Background and Aims: Hepatocyte transplantation has been emerging as a promising alternative treatment modality for acute liver failure; however, marginal quality of cellular transplants is a major limitation. Mesenchymal stem cells (MSCs) could improve liver-specific metabolism of co-cultured hepatocytes. The present work aimed to potentiate hepatotrophic effects of MSCs co-culture using hypoxic preconditioning (HPc).
Methods: Human adipose-derived MSCs were subjected to 20%> (normoxia-preconditioned, NPc) or 2%> O_2 (HPc) for 24 h and co-cultured with primary human hepatocytes in a 3-dimensional architecture (Figure 1, the arrowheads indicating hepatocytes), with mono-cultured hepatocytes as control. Albumin secretion and CCK18 release were assayed to investigate trophic and antiapoptotic effects of co-culture on hepatocytes. Indirect co-culture was established to investigate the role of paracrine factors in hepatotrophic effects of co-culture. Reactive oxygen species (ROS) activity was antagonised by adding N-acetylcysteine to investigate whether HPc potentiated MSCs by intracellular ROS-dependent mechanisms, including TNF-α, TGF-β1, extracellular collagen, and apoptosis-associated caspase and BAX/BCL-2 signalling pathways.
Results: HPc significantly potentiated albumin secretion (day 7, HPc *vs.* NPc, 3.9±0.2 *vs.* 3.3±0.3 folds of control, $P < 0.01$) but further inhibited CCK18 release (day 5, 0.44±0.06 *vs.* 0.63±0.08 fold of control, $P < 0.01$) from co-cultured hepatocytes. Indirect co-culture showed no significant hepatotrophic effects. HPc-induced potentiative effects were eliminated by ROS antagonisation (albumin, 0.83±0.06 fold of HPc co-culture, $P < 0.01$; CCK18, 1.4±0.2 folds of HPc co-culture, $P < 0.01$). Decreased hepatocyte autocrine TNF-α, increased MSCs autocrine TGF-β1 and enhanced MSCs deposition of extracellular collagen mediated potentiative effect of HPc on MSCs co-culture. Enhanced inhibitive effect of HPc on apoptosis of hepatocytes co-cultured with MSCs resulted from downregulated expression of *CASP9*, *BAX*, *BID* and *BLK* and upregulated expression of *BCL-2*.
Conclusions: HPc potentiated MSCs and improved co-culture hepatotrophic and antiapoptotic effects by nonparacrine, intracellular ROS-dependent mechanisms, including autocrine TGF-β, extracellular collagen and caspases and BAX/BCL-2 signalling pathways.



P1299

PLATELET ALPHA-GRANULE RELEASE IN LIVER REGENERATION AFTER HEPATECTOMY

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Background and Aims: Platelet activation results in α-granule exocytosis and release of mediators crucial for various (patho) physiological processes like angiogenesis and liver regeneration. As α-granules contain pro- and anti-proliferative factors, it has been speculated, that platelets are capable of selective α-granule release.
Methods: We investigated if this selective release also occurs *in vivo* and correlates with clinical outcome by monitoring 130 patients undergoing partial hepatectomy.
Results: Liver resection resulted in elevated plasma levels of α-granule factors in all patients. Low plasma levels on the first postoperative day of vascular endothelial growth factor (VEGF) and fibrinogen but high thrombospondin-1 (TSP-1), platelet factor-4 (PF4) and transforming growth factor-β (TGF-β) levels predicted liver dysfunction. Furthermore, platelets of patients with liver dysfunction failed to secrete intra platelet VEGF after liver resection. Based on these results, patients within a high risk group (high TSP-1 and low VEGF) suffered from a significantly increased incidence of postoperative liver dysfunction as well as severe morbidity.
Conclusions: This indicates that the postoperative profile of circulating platelet-derived factors correlates with the ability of the remnant liver to regenerate. Accordingly, the modulation of platelet α-granule release in patients may represent an attractive target for therapeutic interventions to modify liver regeneration or angiogenesis.

P1300

SPHEROID RESERVOIR BIOARTIFICIAL LIVER TREATMENT INHIBITS ALPHA-AMANITIN INDUCED FULMINANT HEPATIC FAILURE IN RHESUS MONKEY MODEL

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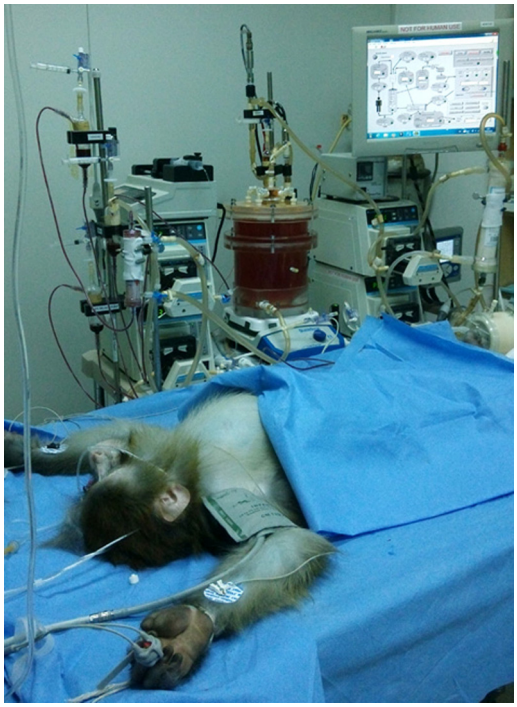
Background and Aims: High mortality rate in amanita phalloides intoxications is principally a result of the fulminant hepatic failure (FHF) following massive death of liver cells due to hepatocellular uptake of α-amanitin (α-AMA), the major amatoxin, with limited therapeutic options. Recovery would be more frequent if a supportive therapy were available to correct the toxic milieu of FHF to prevent its extrahepatic manifestations and to assist in liver regeneration. Therefore, a novel supportive therapy, the Spheroid Reservoir Bioartificial Liver (SRBAL) composed of over 100 gram porcine primary hepatocyte aggregates ("spheroids"), was developed. The spheroids were engineered by a novel rocked high-density suspension culture technique. Once formed, spheroids are placed in a continuous perfusion bioreactor, which provides functionality to the device.
Methods: The SRBAL was recently evaluated in an ambulatory primate model of α-AMA and LPS combine-induced FHF. Rhesus monkeys were randomized into three treatment groups: no therapy (n = 3), no cell device therapy (n = 3), and SRBAL therapy (n = 3). SRBAL treatment was 6 hours in duration after toxin administration

12 hours. There was no difference in the level of liver failure between groups prior to the initiation of treatment.
Results: All treatment procedures were completed successfully without any adverse reaction. All samples presented negative PERV DNA and RT activity. The levels of antibodies were similar before and after treatment. A significant survival benefit was observed with SRBAL compared to the two control groups (100% vs. 0% vs. 0% at 60 hours after toxin administration, $p < 0.001$). Animals treated with the SRBAL maintained stable plasma ammonia levels during treatment compared to control animals. Relatively low plasma concentrations of S-100 beta protein, as a marker of astrocytic damage, from monkeys with hepatic failure during SRBAL therapy were noted. SRBAL therapy can prevent irreversible brain damage from hepatic encephalopathy via reduce the ICP peak in FHF. The clinical symptoms such as acratia, anorexia and abdominal distension were improved and recovered at 5 days after SRBAL treatment.
Conclusions: Results of this pivotal preclinical study demonstrate that the SRBAL improved survival in a xenogeneic model of amatoxin and endotoxin induced FHF.



P1301
A NOVEL ROLE FOR KRÜPPEL-LIKE-FACTOR 6 IN HEPATIC REGENERATION AND ACUTE LIVER FAILURE
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Background and Aims: The zinc finger protein Krüppel-like factor 6 (KLF6) is a multifunctional transcription factor and tumor suppressor. Previous studies identified KLF6 as a mediator of hepatocyte glucose and lipid homeostasis and downregulation of KLF6 was associated with accelerated tumor-growth of hepatocellular cancer. So far, no data is available on the role of KLF6 in regeneration after acute liver injury. Here, we investigated the expression of hepatocyte KLF6 in patients with acute liver failure (ALF), in a human ex-vivo perfusion model of acetaminophen (APAP) induced liver injury and in mice undergoing partial hepatectomy (PHx).
Methods: KLF6 expression was assessed by immunohistochemistry (IHC) in liver samples from patients with drug-induced ALF (n = 10) compared to controls (n = 10). In an established ex-vivo perfusion model non-cirrhotic liver tissue from patients who received partial liver resections for metastatic surgery (n = 6) was treated with APAP for up to 30 hours. KLF6 expression was quantified before and after APAP treatment by qRT-PCR and IHC. In a murine model of hepatocyte specific KLF6 knockout (KO) compared to wildtype (wt) mice we performed PHx (n = 6 mice/group). Utilizing Affymetrix Array (HT-MG430) Genearray and qRT-PCR, we assessed mRNA expression of different targets following PHx. Hepatocyte proliferation after PHx was determined by PCNA and Ki67 staining.
Results: IHC in ALF patients revealed significant upregulation of KLF6 within hepatocytes compared to controls. APAP perfusion of liver tissue significantly induced KLF6 mRNA- and protein-expression. In mice PHx led to significant induction of KLF6 expression at different timepoints. In hepatocyte specific KLF6-KO mice cell proliferation was significantly induced at early timepoints following PHx. The mRNA expression of autophagy-related molecules atg7 and beclin1 was significantly reduced in KLF6-KO before and after PHx compared to wt-animals.
Conclusions: Our findings suggest an important role for KLF6 in liver regeneration, as KLF6 expression is upregulated in different models of acute liver injury and ALF patients. Hepatocyte proliferation following PHx was induced in KLF6-KO, suggesting a role for KLF6 in hepatic regeneration. Reduced atg5 and beclin1 expression suggests a novel link between KLF6 and autophagy.

P1302
EVIDENCE FOR THE ICG-CLEARANCE-TEST AS A RELEVANT PREDICTOR OF POST HEPATECTOMY LIVER FAILURE
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Background and Aims: Liver resection has gained importance in the treatment of liver tumors. In this context, the most significant factor determining postoperative outcome of these patients is the ability of the remnant liver to regenerate. To pursue liver function in an adequate manner, sufficient and clinically viable tests are required. Given the controversially discussed data about the dynamic liver function tests, no consensus about the quantity of the ICG-Test has yet formed. Accordingly, we investigated if the ICG-Clearance test eligible determines post hepatectomy liver failure and clinical outcome.
Methods: Therefore, 130 patients undergoing liver resection surgery were included in this study and ICG-values were recorded one day prior to surgery, as well as on the first and fifth postoperative day. Serum markers of liver function were used to evaluate liver regeneration (LR) and liver dysfunction (LD).
Results: Plasma diffusion rate (PDR), as well as the retention rate after 15 minutes (R15), significantly deteriorated after liver resection ($p < 0.05$) and remained worsened up to the fifth postoperative day. Accordingly, preoperative reduced ICG-levels were able to predict postoperative liver dysfunction (LD) in a sufficient way (AUC [area under the curve] = PDR: 0.72, R15: 0.73; $p \geq 0.05$). Furthermore, we validated that a PDR of <17% and

a R15 of <8% were associated with an increased incidence of postoperative LD (specificity: 82%, sensitivity: 50.5%) and were able to identify high-risk patients prior to surgery.
Conclusions: These findings indicate that the ICG-Clearance Test is a valuable tool for the prediction of LD and poor clinical outcome after liver resection. Additionally, PDR and R15 may represent useful parameters to identify high-risk patients prior to surgery that require consideration and close monitoring for potential complications.

P1303
HUMAN PLASMA TOXICITY IN DIFFERENTIATED HepaRG PROGENITOR CELLS IN THE CONTEXT OF THE BIOARTIFICIAL LIVER

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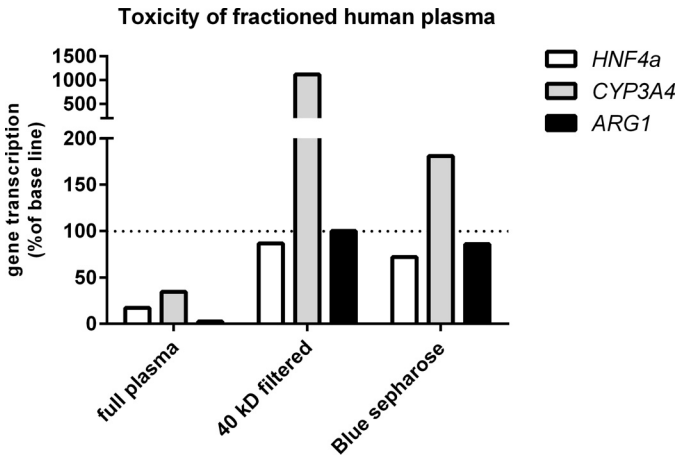
Background and Aims: The AMC-Bioartificial Liver (BAL) is based on the human liver progenitor cell line HepaRG, cultured in 3D under 40% O_2, and is intended to treat patients suffering from Acute Liver Failure. During therapy, cells are in contact with the patients plasma. We previously found that plasma of both healthy and ischaemic-liver rats reduced hepatic transcript levels and hepatic functionality. However, no data on human plasma are available.
We aimed to assess human plasma toxicity on HepaRG cells in monolayer and in the AMC-BAL and to identify the underlying mechanism.
Methods: Cells were cultured in lab scale BALs, culture plates (2D in 20 and 40% O2) and non-woven polyester matrices (3D in 20% O_2). Cultures were exposed to pooled full human plasma from healthy donors or to standard culture medium for 16 hours unless stated otherwise. Transcript levels of hepatic genes Cyp3A4, HNF4A and ARG1 were analysed by qRT-PCR. This panel of genes was previously found to reflect detrimental plasma effects in a consistent and sensitive way. Ammonia elimination and urea production were quantified by biochemical assays as a measure of hepatic functionality. Plasma fractionation was performed with commercial molecular weight-exclusion filters, Bligh & Dyer (B&D) lipid extraction and Blue Sepharose affinity chromatography.



Results: Human plasma had a dose-dependent toxic effect on HepaRG cells. In monolayers we observed an 80% reduction of hepatic gene transcription within 8 hours of plasma exposure. Hepatic functions decreased within 16 hours. The toxic effect was ameliorated when plasma was passed through filters >40 kDa, or run over Blue Sepharose. After B&D extraction, the hydrophobic phase had a toxic effect similar to full plasma.
In BALs, the 16 h plasma exposure effects on transcript levels were less pronounced and functions were unaffected. Cells were also less affected by plasma when cultured under 3D conditions; increased oxygenation had no significant effect.
Conclusions: Plasma toxicity is due to one or more toxic components. The causative agent(s) is/are likely hydrophobic and protein bound, or, if not protein bound, the agent(s) is/are sized >40 kDa.
HepaRG cells are partly protected from toxicity when cultured in the AMC-BAL, 3D configuration contributes to this effect but is not the single explanatory factor. Practically, these findings suggest that it may be beneficial to combine BAL support with artificial liver support, such as albumin dialysis, to maintain high hepatic functionality during therapy.

P1304
PREDICTION OF INTRACRANIAL HYPERTENSION IN PATIENTS WITH ACUTE LIVER FAILURE

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Background and Aims: Acute liver failure (ALF) is a life threatening condition that in combination with persistent hyperammonaemia carries a high risk of intracranial hypertension (ICH). The pathophysiology involved is believed to be brain oedema and hyperaemia due to cerebral metabolic disturbances. In this study we aimed to construct a predictive model identifying patients who develop ICH from a set of clinically available baseline variables in a cohort of patients from our liver intensive care unit.
Methods: 45 patients with ALF and persistent hyperammonaemia *or* clinical signs of ICH were included. Cerebral microdialysis and continuous measurement of intracranial pressure (ICP) was performed. Baseline values of ICP, arterial lactate and ammonia were registered. The concentration of lactate, pyruvate and glutamine was measured in the microdialysate. The need for renal replacement therapy (RRT) and vasopressors at inclusion was registered. ICH was defined as ICP >20 mmHg for more than one hour. As predictive functions, we used logistic regression and support vector machines and evaluated the prediction error rate of ICH/non-ICH with five-fold cross validation.
Results: 21 patients (47%) developed ICH 39 (0–108) hours [median (range)] after inclusion and was the cause of death in ten of those patients. The baseline arterial ammonia was 186 (60–340) μM, the cerebral lactate to pyruvate ratio 29 (9.1–173) and glutamine 3.2 (0.2–7.3) mM. Only glutamine was significantly different between subjects with and without ICH (3.7 vs. 2.7 mM, respectively, $p < 0.05$). A support vector machine model based on baseline ICP and microdialysate analysis gave the lowest prediction error rate (30%).
Conclusions: ICH is a frequent complication in this subgroup of ALF patients and is associated with elevated extracellular glutamine in the brain as previously reported. The median lactate to pyruvate ratio was close to normal levels. Our predictive model based on neuroinvasive observations at baseline allowed us classify patients as ICH/non-ICH correctly in 70% of the cases.

P1305
MITOCHONDRIAL FUNCTION IS PRESERVED IN CEREBRAL CORTEX IN A RAT MODEL OF ACUTE HYPERAMMONAEMIA AND SYSTEMIC INFLAMMATION

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Background and Aims: In acute liver failure (ALF) cerebral oedema and high intracranial pressure (ICP) are potentially

deadly complications. Astrocytes cultured in ammonia have shown mitochondrial dysfunction and in rat models of liver failure lactate production in the brain has been observed. These findings support the hypothesis of a compromised brain metabolism during ALF. In contrast, normal lactate levels are found in cerebral microdialysate of ALF patients and the oxygen to glucose ratio of cerebral metabolic rates remains normal. To investigate this inconsistency we studied the mitochondrial function in brain tissue with respirometry in an animal model of acute hyperammonaemia and systemic inflammation as well as in a dose-response study.

Methods: Wistar rats were given ammonia+lipopolysaccharide (LPS) or saline+saline. Ammonia or saline was infused for 120 minutes before the animals were sacrificed. Cerebral cortex was analysed with respirometry (Oroboros Instruments). Cortex homogenate was studied in a chamber measuring the oxygen consumption rate. Substrates of the citric acid cycle, uncouplers and inhibitors of the mitochondrial complexes were successively added to the chamber to investigate the mitochondrial function in detail. In a separate dose-response experiment cortex from healthy rats was incubated for 120 minutes in sodium or ammonium acetate in concentrations up to 80 mM prior to respirometry.

Results: Infusion of ammonia resulted in hyperammonaemia (1550±147 μM vs. control 48±5 μM, $p < 0.01$). This was associated with an elevated ICP (6.8±2.1 mmHg vs. control 2.0±0.4 mmHg, $p < 0.05$) and tissue lactate concentration (23±5 mM vs. 16±0.4 mM, $p < 0.05$). No differencesbetween the groups was found in the total respiratory capacity or the function of the individual mitochondrial complexes. We did not observe increased proton leak across the inner mitochondrial membrane. Ammonia in concentrations of 40 and 80 mM reduced the respiratory capacity but not compared to corresponding sodium acetate controls. We found a linear relationship between the inner membrane proton leak and the ammonia/sodium acetate concentration.

Conclusions: Hyperammonaemia and systemic inflammation lead to increased ICP and cerebral lactate accumulation. We found no indications of impaired oxidative metabolism. In the dose-response study, extreme concentrations of ammonia lead to reduced oxidative capacity which however also was found after exposure to sodium acetate suggesting an effect of osmolality rather than ammonia itself.

P1306
SYPHILITIC HEPATITIS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)-INFECTED PATIENTS

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Background and Aims: Hepatitis secondary to *Treponema pallidum* infection was reported as an uncommon presentation of early syphilis. Resurgence of syphilis has been observed among men having sex with men (MSM) infected with HIV. Data regarding the prevalence and pattern of syphilitic hepatitis among HIV-infected patients is limited.

Methods: Retrospective analysis of epidemiological, clinical and laboratory data of HIV-infected patients diagnosed with early syphilis in the TASMC from January 2000 to March 2014.

Results: 125 cases of primary or secondary syphilis were diagnosed in 116 HIV-infected patients. All the subjects were male with a median age of 37 years. 96% were MSM. Syphilis and HIV were diagnosed simultaneously in 21.6%, and 52% were receiving ART at the time of diagnosis. Liver involvement was observed in 59 cases (47.2%). Comparing patients with syphilis to those with syphilitic hepatitis there was no difference regarding mean age, risk factors, duration of HIV prior to syphilis diagnosis, ART treatment or HIV viral load. A significant decrease was observed in mean CD4 and CD8 cell count in the syphilitic hepatitis group (for CD4: 610.9±248.7 cells/mm^3 vs. 471.3±195.9 cells/mm^3, $p = 0.001$; for CD8: 1112.8±537.8 cells/mm^3 vs. 874.8±442.9 cells/mm^3, $p = 0.01$) as well as a tendency towordes higher VDRL titers ($p = 0.06$). The pattern of liver enzymes elevation was mixed in 51% of cases, predominantly cholestatic in 37%, and predominantly hepatocellular in 13%. Elevation of cholestatic enzymes was mild (<2.5 times of ULN) in 47.5%, however 15.3% of the cases presented with a marked increase (10 x ULN). Most patients had either no or only mild elevation of hepatocellular enzymes (37.3% and 32.2% of the cases respectively). However 11.9%, presented with at least moderate hepatocellular enzyme elevation (>5 times of ULN). Symptomatic and biochemical resolution of the hepatitis ensued following antibiotic therapy in all patients.

Conclusions: Hepatitis is common in HIV infected patients with syphilis and may be the presenting sign of *Treponema pallidum* infection. Liver impairment was predominantly cholestatic, but transaminases elevations were not uncommon. Syphilis must be considered in the diagnosis of HIV patients presenting with hepatitis.

P1307
INHIBITION OF PANNEXIN1 CHANNELS ALLEVIATES ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN MOUSE

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Background and Aims: Pannexins (Panx) constitute a relatively new family of transmembrane proteins that form channels linking the cytoplasmic compartment with the extracellular environment. The pannexin family consists of 3 members of which Panx1 is expressed in the liver. It is well-known that Panx1 plays a key role in innate immunity by facilitating activation of the inflammasome. Recently, Panx1-mediated inflammation was found to be involved in non-alcoholic steatohepatitis in mouse. In the present study, it is investigated whether this also holds true for acute liver failure *in casu* triggered by acetaminophen.

Methods: Mice were overdosed with acetaminophen followed by treatment with the Panx1 channel inhibitor ^{10}Panx1 after 1.5 hours. Sampling was performed 24 hours after acetaminophen administration. Evaluation of the effect of Panx1 channel inhibition was based on a number of clinically relevant read-outs, including assessment of alanine and aspartate aminotransferase serum levels as well as histopathological examination of liver tissue with quantification of cell death. Inflammation was studied by measurement of cytokine levels in liver and serum.

Results: All parameters measured indicate a significant reduction of liver damage, including cell death and inflammation, upon treatment of acetaminophen-intoxicated mice with ^{10}Panx1.

Conclusions: The results of this study demonstrate the clinical potential of Panx1 channel inhibition as a therapeutic strategy in the treatment of acetaminophen-induced acute liver failure.

P1308

ROTATIONAL THROMBOELASTOMETRY CAN AVOID UNNECESSARY SUBSTITUTION OF COAGULATION FACTORS IN ACUTE AND CHRONIC LIVER DISEASES

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Background and Aims: Acute liver failure (ALF) and liver cirrhosis (LC) are associated with complex hemostatic disorders. Conventional tests for blood clotting (International Normalized Ratio, INR; partial thromboplastin time, PTT; number of thrombocytes; fibrinogen) would suggest increased bleeding tendency. Though, many patients exhibit increasing incidence of thrombosis with liver disease progression. We aimed to elucidate, if rotational thromboelastometry (ROTEM®) can reduce substitution of coagulation factors to patients with ALF or LC prior intervention.
Methods: Patients with ALF with (n = 10) or without ROTEM® (n = 9) and patients with LC with (n = 25) or without ROTEM® (n = 16) analysis prior an intervention were analysed retrospectively. All recruited patients exhibited compromised coagulation according to conventional coagulation tests. Substitution of coagulation factors was assessed and for patients not receiving substitution, theoretical substitution according to conventional standards was calculated.
Results: Both groups of ALF patients (with and without ROTEM®) had impaired coagulation according to conventional parameters. Quick, INR, PTT, platelet count, and fibrinogen concentration did not differ between the groups. 4 patients without ROTEM® measurement received 2000 IU PCC (prothrombin complex concentrate) and 1 patient received 6000 IU PPC. In patients with ROTEM® analysis no substitution of coagulation factors was performed, as this was not indicated. Based on conventional coagulation parameters, these patients would have received a mean amount of 1150 IU PPC, corresponding to approx. 9200€. All LC patients exhibited impaired coagulation and both groups had roughly equal coagulation parameters. Patients in the group without ROTEM® received a total of 20500 IU PCC, 12U fresh frozen plasma (FFP) and 1g fibrinogen. In contrast patients with ROTEM® were given 8500 IU PCC, no FFP, and 18g fibrinogen. Based on conventional parameters only these patients would have received 37000 IU PCC and only 8.5g fibrinogen. In total the reduction of substitution would amount to approx. 20,600 €. In none of the patient groups adverse events or complications were observed.
Conclusions: By ROTEM® analysis hemostasis can be evaluated in more detail for ALF and in patients with chronic liver diseases. Substitution of coagulation factors can be significantly reduced without increasing the risk for the patients.

Cirrhosis and its complications: a. Pathophysiology

P0082

CD34 PATTERNS IN BONE MARROW INDICATE THE STATUS OF FUNCTIONAL RESERVE OF BONE MARROW IN CIRRHOSIS

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Background and Aims: Cirrhosis can cause various systemic manifestations. Bone marrow microenvironment alterations in the cirrhosis are not clearly known. Present study was aimed to assess the bone marrow profile in cirrhosis cases.
Methods: Bone marrow (BM) biopsies and aspirate of cirrhosis cases were analyzed. Bone marrows were performed to evaluate cytopenias and pyrexia of unknown origin. These were assessed for cellularity, myeloid: erythroid ratio, myelogram, iron and reticulin. Hematological, clinical parameters and liver severity indices were correlated. Immunohistochemical staining for CD34, Thy-1, S-100, beta catenin, notch-1 and p53 were done for the assessment of in bone marrow cellular microenvironment. Two pathologists independently evaluated the sections. Positive stained cells were counted minimum in five high power fields and average was calculated.
Results: A total of 96 cases of bone marrows were studied. Mean age was 49±11.8 years and male:female ratio was 2:1. On examination of BM biopsy sections two patterns of CD34 staining were noted: (1) Cellular CD34 (*cCD34*: cells >10/20×, with occasional vessels <2 capillaries/20×); in 43 and (2) Vascular CD34 (*vCD34*: vessels >10/20× with <5/20× cells positivity); in 53 cases. Mean age in both groups were comparable (p = 0.146). Hemoglobin (p < 0.001), platelet counts (p = 0.004) and total albumin (p = 0.001) were significantly lower in the *vCD34* group than in the *cCD34* group. Spleen size was comparable in both groups (p = 0.961). Modified end stage liver disease score [MELD] (p < 0.001), total bilirubin (p < 0.001), international normalized ratio [INR] (p = 0.001), myeloid:lymphoid ratio (p = 0.05) and TNF-α (p = 0.013) were significantly higher in the *vCD34* group. In the *vCD34* group 27/53 showed reticulin lying down, whereas in the *cCD34* group only 5/43 had reticulinization of the marrow. Immunohistochemical staining showed Thy-1 positive hematopoietic stem cells were more evident in *cCD34* (21.4±4.1) than in *vCD34* (3.8±1.6) (p < 0.001). Similarly S-100 positive Schwann cells were much higher in *cCD34* (15.2±5) as compared to *vCD34* (4.7±1.8) (p = 0.03). Results are summarized in Table 1.
Conclusions: Bone marrow in cirrhosis has two distinct patterns. *cCD34* pattern in bone marrow indicates adequate reserve for hematopoietic activity and seen with favorable liver functions. *vCD34* points towards the depletion of the bone marrow reserves. Elevated myeloid:lymphoid ratio and increased reticulin in the *vCD34* group denote ageing of BM.

Table 1. Comparison of parameters in Cellular CD34 (*cCD34*) and Vascular CD34 (*vCD34*) groups

Parameter	Cellular CD34 (n = 43)	Vascular CD34 (n = 53)	P value
Age (years)*	48±12.5	50.5±11.2	0.176
Male:Female	1.8:1	2.1:1	0.328
Spleen size (cm)*	14.2±2.7	14.8±3.7	0.961
Hemoglobin*	9.9±2	8.4±1.9	0.001
Total leukocyte count $\times 10^3$/cu.mm**	4 (3–7)	5 (3.5–9)	0.148
Platelet count $\times 10^3$/cu.mm**	89 (59–180)	64 (42–90)	0.004
Myeloid:Erythroid ratio*	1.2±0.7	1.2±0.8	0.981
Cellularity (%)*	53.1±11.3	48.2±14.1	0.084
Bone marrow fibrosis; reticulin positive (%)	11.6 (5/43)	51 (27/53)	0.01
Bilirubin (mg/dl)**	1 (1–3)	4 (3–5.7)	<0.001
Creatinine (mg/dl)	1.2±0.8	1.5±0.7	0.310
Albumin (g/dl)	3±0.7	2.4±0.6	0.001
International Normalized Ratio**	1 (1–2)	2 (1–2)	0.001
Modified End stage Liver Disease score*	13.8±6.7	21.1±8.2	<0.001
Myeloid:Lymphoid ratio	3.9±2.3	5.2±3.8	0.05
Lactate dehydrogenase [LDH] (IU/L)*	580±255.2	751.8±437	0.380
Serum ferritin (μg/L)**	182 (73–1638)	245 (44–851)	0.170
Tumor necrosis factor-α*	4±2.8	20.8±15.2	0.013

Key: *Mean±standard deviation; **Median (interquartile range).

P0083

SODIUM BENZOATE AND RIFAXIMIN ARE ABLE TO RESTORE BLOOD–BRAIN BARRIER INTEGRITY IN CIRRHOTIC RATS WITH HEPATIC ENCEPHALOPATHY

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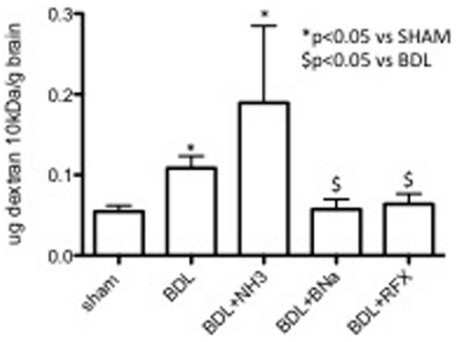
Background and Aims: Hepatic encephalopathy (HE) is a severe complication of cirrhosis which independently influences prognosis. We previously showed an increase in blood–brain barrier (BBB) permeability in cirrhotic rats with HE. The aim of the present work was to assess the effects of sodium benzoate (Bna), a drug removing ammonia through non-urea cycle pathway, and Rifaximin (RFX), a non adsorbable antibiotic, on BBB permeability in cirrhotic rats with HE.

Methods: Three groups of rats were considered: SHAM, Bile Duct Ligation (BDL), BDL + hyperammonic dietary (BDL-NH3). In each group, rats were treated by BNa or RFX. HE was assessed using neurocomportemental testing (6 minutes tail suspension test assessing the time of immobility). NH3 levels were assessed before sacrifice. BBB permeability was assessed by IV injection of a fluorochrome (Texas Red 10kDa) before transcardial washing. Brain fluorescence was estimated by fluorimetry after right hemisphere squeezing.

Results: Mean time of immobility was longer in BDL-NH3 and BDL rats than in SHAM ($p = 0.0004$). Ammonemia was significantly higher in the BDL-NH3 than in BDL rats, and higher in the BDL than in SHAM rats ($p < 0.0001$). Intra-cerebral fluorescence was significantly higher in BDL-NH3 than in BDL group, and higher in BDL than in SHAM group ($p = 0.029$) confirming the passage of the fluorochrome through the BBB. BNa treatment significantly decreased ammonemia levels and intra-cerebral fluorescence in the BDL and BDL-NH3 rats ($p < 0.04$ for all) but did not modify the mean time of immobility. On the contrary, RFX treatment did not modify ammonemia levels but significantly decreased intra-cerebral fluorescence ($p < 0.05$) and the mean time of immobility ($p = 0.0004$) (see Figure).

Conclusions: In cirrhotic rats displaying HE, BBB permeability is increased, through different mechanisms dependent and independent of hyperammonemia. BNa and RFX are effective in restoring BBB integrity in HE cirrhotic rats but only RFX is able to decrease HE in this model



P0084

OXIDATIVE STRESS REDUCTION BY NANOPARTICLES OF CERIUM OXIDE (CeO_2NPS) PARTIALLY REVERTS THE ACTIVATION OF PORTAL ENDOTHELIAL CELLS FROM CIRRHOTIC RATS

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Background and Aims: Hepatic and splanchnic endothelial cells undergoes phenotypic transformation process during the progression of liver fibrosis. However, it is unknown whether this endothelial dysfunction occurs in other vascular areas such as the portal vein. The aim of this study was, therefore, to characterize the degree of activation of endothelial cell lines isolated from the portal vein of control (PVEC-CT) and cirrhotic (PVEC-CH) rats and evaluate new strategies to block the activation of PVECs.

Methods: Cirrhosis was induced in male Wistar rats by inhalation of CCl_4. The PVECs were isolated from the portal vein using anti-CD31 antibodies. The phenotypic differences between PVEC-CT and PVEC-CH were evaluated by RT-PCR. CeO_2NPs were synthesized by precipitation of cerium salt and tetramethylammonium hydroxide.

Results: Primary cultures of PVEC-CT and PVEC-CH (purity >95%, two clones per condition) were immortalized by retroviral transduction of the SV40 virus T antigen. The PVEC-CH showed an overexpression of collagen-I, endothelin-1, TIMP1, TIMP2, IL-6 and PLGF ($n = 10$), compared with PVEC-CT. The same panel of genes was overexpressed in isolated portal veins of CH rats ($P < 0.01$, $n = 10$ per condition). In order to find common causes for this phenotypic alteration, we studied the degree of oxidative stress present in these cell lines. The PVEC-CH showed a significant increase of oxidative stress (dichlorofluorescein, $n = 10$) compared with PVEC-CT. Several pre-clinical studies have demonstrated the antioxidant properties of CeO_2NPs. Consistent with these studies, the treatment of PVEC-CH with 1 μg/mL CeO_2NPs for 24 hours significantly reduced the levels of oxidative stress and the expression of TIMP2 and IL-6 genes ($n = 10$). In addition, we studied the therapeutic potential of CeO_2NPs in vivo. Cirrhotic rats were treated i.v. with vehicle ($n = 5$) or 0.1 mg/kg CeO_2NPs ($n = 5$) twice a week for 2 weeks. The portal veins of CH rats treated with CeO_2NPs showed a significant decrease of IL-6, compared to the vehicle treatment.

Conclusions: We generated immortalized cell lines of PVECs from CT and CH rats that maintain their phenotypic differences. Intracellular oxidative stress reduction, mediated by CeO_2NPs treatment, partially reverted the pathological phenotype of PVECs from cirrhotic rats *in vivo* and *in vitro*.

These results may open new perspectives for the applicability of nanomedicine in the treatment of endothelial dysfunction.

P0085

HEPATIC ENCEPHALOPATHY: CEREBROSPINAL FLUID METABOLOMICS HIGHLIGHTS ALTERATION OF MULTIPLE METABOLIC PATHWAYS REPRESENTING NEW POTENTIAL THERAPEUTIC TARGETS

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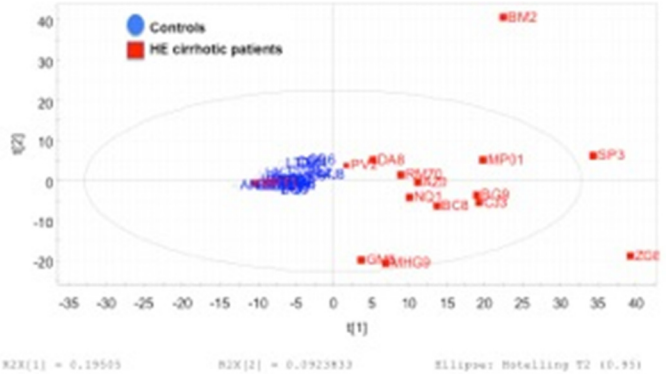
Background and Aims: Hepatic encephalopathy (HE) is a neurological complication of cirrhosis, impairing survival and quality of life. Its incidence is growing because of the improved prognosis of other complications of cirrhosis, and of the widespread use of TIPS. However, besides hyperammonemia which is often pointed out as a cause of HE, the pathophysiological mechanisms of HE remains poorly understood, which prevents the development of therapeutic strategies. To address this issue, metabolomics was used to identify dysfunction of metabolic pathways in cerebrospinal fluid (CSF) samples of cirrhotic patients suffering from HE. The aim of this study was to detect new therapeutic targets for HE associated with cirrhosis.
Methods: Cerebrospinal Fluid (CSF) samples were collected on 14 cirrhotic patients admitted in ICU for HE, in whom infection of central nervous system has to be ruled out, and were compared to CSF of 27 control patients without any proven neurological disease. Metabolomic analysis was performed using 3 liquid chromatographies coupled to high resolution mass spectrometry methods (LC-HRMS). Informatic data processing tools were used.
Results: LC-HRMS methods led to the characterization of 150 metabolites in CSF samples of HE patients, which were mainly aminoacids and organic acids. Interestingly, according to human metabolome database, 40% of those metabolites were not reported as present in CSF before. HE patients could be easily discriminated from controls on the basis of metabolomic information (Figure). Concentrations of 102 metabolites were found to be significantly altered in HE patients: metabolotypes displayed alterations in several major metabolite classes such as ammoniac, bile acids, but also amino-acids, acylcarnitines, and nucleosides. Accumulations of acetylated compounds, which could be due to a defect of the Krebs cycle, were reported for the first time in HE patients, and could constitute interesting therapeutic targets.



Conclusions: By enabling the simultaneous monitoring of a large set of metabolites in cirrhotic patients with HE, CSF metabolomics highlighted several altered metabolite pathways linked to ammonia metabolism, neurotransmission and energy metabolism. The pharmacological relevance of our findings has to be explored on animal models, as they could constitute interesting new therapeutic targets.

P0086

HYPERAMMONEMIA ALTERS AQP4 AND MIR-196A EXPRESSION IN PRIMARY ASTROCYTES: IMPLICATIONS FOR BRAIN EDEMA

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Background and Aims: A key feature accounting for hepatic encephalopathy (HE) symptoms is brain oedema. During HE, NH_4^+ significantly increases in the general circulation leading to a higher astrocyte glutamine synthesis, increasing the intracellular osmotic pressure. To counteract it, astrocytes promote water inwards movement through aquaporin channels (AQPs). In mammals, AQP expression is regulated at the transcriptional and post-transcriptional level by different signals, including ammonia and small nucleic acids. Here, we aim to characterize the role of miR-196a in AQP4 gene expression control during hiperammonemic conditions.
Methods: Astrocyte culture was prepared from post-natal CD1 pup mice using trypsin. Cells were grown in DMEM supplemented with FBS (10%) at 37°C under 5% CO_2 atmosphere, and were exposed to 5 or 10 mM NH_4Cl solution. RNA was extracted to synthetize cDNA to be used in qPCR for AQP4, GFAP, and other transcripts involved in the cellular volume response. Cytotoxic effects of NH4Cl were determined using the MTT assay. Cell volume was determined using Calcein-AM (4μM). The cell monolayer was optically sectioned along the vertical axis using confocal microscopy and the Zen-lite software. Images were using the 3-D counter tool of the Fiji version ofImageJ software v1.48. Volume is expressed as the mean volume in μm^3.
Results: To determine the viability of NH_4Cl treated cells, MTT assays were performed. Treatment of astrocytes with NH_4Cl does not modify viability (96±3.5% *vs* control cells). In accordance, NH_4Cl significantly increases the expression of AQP4 (1.4±0.1 fold change *vs* control) at 12 hr of treatment, while that of GFAP was increased just after 24 hr of treatment (1.8±0.3 fold change *vs* control), as determined by qPCR and Western blot assays. Moreover, when AQP5 and APQ9 transcripts were analysed, a significant increased was also observed. Cell volume status indicates that astrocytes incubated with NH_4Cl, increase its volume in a significant manner (301963±30056 μm3 *vs* control cells). We measured the miR-196a expression and found that miR-196a is strongly down-regulated in response to the NH_4Cl treatment, thus suggesting that might be of relevance for AQP4 gene expression modulation.
Conclusions: NH_4Cl treatment increases the expression of AQP4 and GFAP, in a dose-dependent manner. This increase is accompanied by a reduction in the miR-196a expression, strongly suggesting that this miRNA is involved in the control of cellular volume response.

P0087
LOW SERUM C24 CERAMIDE IS PREDICTIVE OF HEPATIC DECOMPENSATION AND POOR OVERALL SURVIVAL IN LIVER CIRRHOSIS

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Background and Aims: Very long chain ceramides constitute bioactive molecules with functional implications in liver homeostasis. Particularly, ablation of very long chain ceramides in a ceramide synthase 2 knockout mouse model has been shown to cause a severe hepatopathy.
Methods: We assessed via mass spectrometry serum concentrations of sphingolipid metabolites in a series of 244 patients with liver cirrhosis prospectively followed for a median period of 228±217 days.

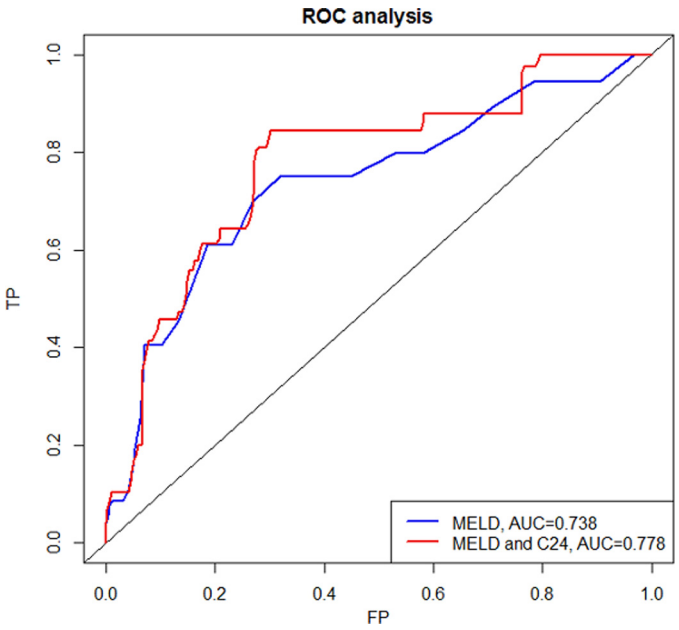

Figure 1.

Results: We observed a significant decrease of long and very long chain ceramides, particularly of C24ceramide, in patients with increasing severity of liver cirrhosis ($p < 0.001$). Additionally, hydropic decompensation, defined by clinical presentation of ascites formation, was significantly correlated to low C24ceramide levels ($p < 0.001$) while a significant association to hepatic decompensation and poor overall survival was observed for low serum concentrations of C24ceramide ($p < 0.001$) as well. Multivariable Cox regression in patients with decompensated liver cirrhosis further identified low serum C24ceramide as an independent predictor of overall survival [hazard ratio (HR) = 0.411, confidence interval (CI) 0.144–1.171, $p = 0.096$]. A novel updated prediction score combining Model of end stage liver disease (MELD) and C24ceramide showed a higher area under the curve (AUC) value as compared to the MELD score alone (0.778 versus 0.738, $p < 0.001$) in a time-dependent receiver operating characteristic (ROC) analysis regarding overall survival in these patients (Figure 1).
Conclusions: Serum levels of long and very long chain ceramides show a significant correlation to disease severity and hepatic decompensation and are independently associated with overall survival in a prospective series of patients with liver cirrhosis. Particularly C24ceramide may be considered as a novel non-invasive biomarker in patients with decompensated liver cirrhosis.

P0088
FERMENTED MILK CONTAINING *Lactobacillus paracasei* subsp. *paracasei* CNCM I −1518 REDUCES BACTERIAL TRANSLOCATION IN RATS TREATED WITH CCl_4

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Background and Aims: Certain probiotics can prevent pathological bacterial translocation in liver diseases by modulating intestinal microbiota and improving gut barrier and immune disturbances. The aim was to evaluate the effect of a fermented milk containing *Lactobacillus paracasei* subsp. *paracasei* CNCM I −1518 on bacterial translocation, intestinal microbiota, gut barrier and inflammatory response in rats with experimental cirrhosis induced by CCl_4 administration.
Methods: Thirty-nine Sprague-Dawley rats treated with CCl_4 were randomized into two groups: a probiotic group ($n = 20$) that received fermented milk containing *L. paracasei* subsp. *paracasei* CNCM I −1518 in drinking water, and a water group ($n = 19$) that received water only. Treatment began at week 6 of CCl_4 administration and continued until laparotomy, performed one week after development of ascites. A control group included 10 healthy rats. At the end of the study we evaluated bacterial translocation, intestinal flora, intestinal barrier (ileal claudin-4, β-defensin-1, occludin and malondialdehyde as index of oxidative damage) and cytokines in mesenteric lymph nodes and serum.
Results: Mortality during the study was similar in the probiotic group (7/20, 35%) and the water group (6/19, 31%) ($p = 1$). The incidence of bacterial translocation was 1/13 (7.7%) in the probiotic group, 7/13 (54%) in the water group ($p = 0.03$ vs probiotic group) and 0/10 in the control group ($p = 0.007$ vs water group). The concentration of ileal and cecal enterobacteria and enterococci was similar in the probiotic and the water groups. The ileal β-defensin-1 concentration was higher and ileal malondialdehyde levels were lower in the probiotic group than in water group ($p = 0.01$). There were no differences in serum cytokines between groups but TNF-α levels in mesenteric lymph nodes were lower in the probiotic group than in the water group ($p = 0.02$).
Conclusions: Fermented milk containing *Lactobacillus paracasei* subsp. *paracasei* CNCM I −1518 decreases bacterial translocation and ileal oxidative damage and increases ileal β-defensin-1 expression in rats treated with CCl_4, suggesting an effect on the intestinal barrier integrity.

P0089
PLASMA BETATROPHIN LEVELS IN PATIENTS WITH LIVER CIRRHOSIS

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Background and Aims: Insulin resistance (IR) is common in cirrhosis and with advancement of liver disease. In these patients there is a compensatory increase in pancreatic B-cell insulin

secretion to overcome the IR. Recently, Douglas A. Melton's group from Harvard University reported the identification of betatrophin as a circulating protein secreted from the liver under insulin resistant states which is sufficient to dramatically and specifically increase the replication of B-cells in mouse resulting in an increased functional B-cell mass over time. In addition, in human studies betatrophin levels are associated with indexes of IR. The role of betatrophin in cirrhosis is at present unknown. The aim of this study was to investigate plasma levels of betatrophin in cirrhosis

Methods: We measured plasma betatrophin concentrations in 40 patients with cirrhosis (27 in Child–Pugh's class B, and 13 in Child–Pugh's class C) and compared these values with those in 15 age-matched healthy subjects. According to MELD score, 20 patients showed a MELD score higher than 14. IR was assessed by the Homeostasis Model Assessment (HOMA).

Results: Plasma betatrophin levels were significantly increased in patients with cirrhosis compared with healthy subjects. In addition, we found a positive correlation between plasma betatrophin levels and severity of cirrhosis according Child–Pugh or MELD score. Moreover, IR in cirrhotic patients was seen in 82.5%. In these group of patients betatrophin levels were significantly higher than group of patients without IR.

Conclusions: Plasma betatrophin is increased in patients with cirrhosis compared with healthy subjects. The increase in plasma betatrophin levels is related to the severity of cirrhosis as well as with the emergence of IR. Thus, these preliminary results show that betatrophin may contribute to counteract at least in part IR in patients with cirrhosis but more studies are needed to confirm it.

P0090

MELATONIN PROTECTS THE LIVER IN AN EXPERIMENTAL MODEL OF CIRRHOSIS

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Background and Aims: Liver diseases are a major public health problem, accounting for a significant number of hospital visits and admissions and an increasing mortality rate. Melatonin (MLT) is a powerful antioxidant molecule that has been shown to be beneficial in various conditions, including liver diseases. The objective of the present study was to evaluate the effect of MLT on experimental liver cirrhosis induced by carbon tetrachloride (CCl_4) in rats.

Methods: We used 20 male Wistar rats (230–250 g) divided into 4 groups: I: Control (CO); II: CO+MLT; III: CCl_4; and IV: CCl_4+MLT. CCl_4 was administered as follows: 10 doses every 5 days, 10 doses every 4 days, and 7 doses every 3 days. MLT was administered intraperitoneally at a dose of 20 mg/kg from the 10th week to the end of the experiment (16th week).

Results: The use of MLT decreased lipid peroxidation (F2-isoprostanes) and NAD(P)H:quinone oxidoreductase 1 (NQO1) expression in the CCl_4+MLT group compared with the CCl_4 group. Liver histological analysis with HE staining showed that the animals of the CCl_4 group had histological changes, such presence of inflammatory infiltrate. In the CCl_4+MLT groups; however, the incidence of presence of inflammatory infiltrate were lower than those of the CCI4 group. The animals of the CCl_4 group, also had significantly increased NF-κB p65 and iNOS expressions when compared with the control groups. The use of MLT caused a significant decrease in the expression of these proteins in the CCl_4+MLT group when compared with the CCl_4 group. CCl_4 significantly increased the expression of TGF-β1 and α-SMA. In contrast, the group receiving MLT significantly decreased the expression of these proteins when compared with the CCl_4 group, thus suggesting the inhibitory effect of MLT on the activation of hepatic stellate cells and ECM deposition. With it animals of the CCl_4+MLT group showed a significant reduction of fibrosis with incomplete fibrotic septa and nodules, observed in the histological staining picrosirius. Liver fibrosis changes liver vascular architecture creating a hypoxic environment, which is an important stimulus for the production of angiogenic factors such as vascular endothelial growth factor. In the CCl_4+MLT group, this expression was significantly reduced when compared with the CCl_4 group.

Conclusions: Our findings suggest that MLT has a potent antifibrogenic effect, modulating parameters of oxidative stress, angiogenesis, and inflammation.

P0091

N-ACETYLCYSTEINE MODULATES ANGIOGENESIS, VASODILATION AND DNA DAMAGE IN STOMACH OF PORTAL HYPERTENSIVE RATS

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Background and Aims: Portal Hypertension is associated with the development of a collateral circulation due to a progressive increase on portal pressure. This hyperdinamic state leads to dilation in several segments of the digestive tract, including stomach. Our aim was evaluate the antioxidant effect of N-Acetylcysteine (NAC) on the stomach of on portal hypertensive rats.

Methods: The animals were divided in four experimental groups (n = 6): Sham-operated (SO), SO + NAC, Partial portal vein ligation (PPVL) and PPVL + NAC. N-acetylcysteine (10 mg/kg ip) was administered daily for 7 days and started 8 days after surgery. We performed evaluation of portal pressure, performed immunohistochemistry of eNOS, VEGF and nitrotyrosine (NTT) proteins in stomach. We also evaluated the expression of endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) by Western blot analysis and assessed DNA damage in blood sample by comet assay. Statistical significance was calculated using Graphpad Instat, version 3.0 for Windows. Variance analysis (ANOVA) and Student-Newman-Keuls were used for multiple analysis, and the level of significance was 5% ($P < 0.05$). For the comet assay, the normality of variables was evaluated using the Kolmogorov–Smirnov test.

Results: The portal hypertensive group showed an increase in portal pressure eNOS, VEGF and NTT expression when compared to SO group. NAC was able to decrease portal pressure values, and the expression of all the proteins evaluated: eNOS, VEGF and NTT when compared to the PPVL group. Furthermore, NAC was able to modulate DNA damage in PPVL + NAC animals.

Conclusions: In conclusion, NAC was able to minimize gastric vasodilation, evaluated by eNOS, angiogenisis, evaluated by VEGF, and oxidative stress evaluated by NTT. This antioxidant treatment protected gastric mucosa also from DNA damage. Due to this results, we believe NAC is able to protect stomach from the alterations developed by the PPVL procedure.

P0092

IL-17 A AND F ISOFORMS AND THEIR RECEPTORS MEDIATE LIVER DAMAGE IN EXPERIMENTAL CHOLESTASIS AND THE IL17 A/F HETERODIMER INDUCES A PROFIBROGENIC PROFILE IN HEPATIC STELLATE CELLS IN VITRO

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Background and Aims: Significant decrease in liver fibrosis in IL-17RA knockout mice has been demonstrated. However, the expression of IL-17 isoforms and their receptors in cholestatic liver fibrosis has not been explored; also, no information are about IL-17 heterodimer on hepatic stellate cells (HSC) signaling in vitro. The aim of this study was to analyze the expression variability of IL-17A, IL-17F and their receptors IL-17RA and IL-17RC in the liver of rats with cholestasis; aditionally we investigate the participation of IL-17 A/F heteodimer on HSC signaling
Methods: Male Wistar rats were sacrificed at days 8 and 30 after Bile duct ligation (BDL). IL-17A, IL17-F, IL-17RA and IL-17RC expression was determined by qRT-PCR in the liver. IL-17 and RORgt, were analyzed in liver homogenates by Western blotting. Activated HSC cells were stimulated with IL-17A/F, then the transcriptional factors Stat-3p, NF-κB and Smad-2p and profibrogenic genes collagen I, III and TGF-b were evaluated by qRT-PCR.
Results: Hepatic gene expression of IL-17A, IL-17-F and IL-17RC dramatically increased at 8 and 30 days post BDL. IL-17RA significantly increased at 30 days post BDL. The overall IL-17RC level was positively correlated with both IL-17A and IL-17F ($r = 0.48$, $P = 0.005$; and $r = 0.39$, $P = 0.012$, respectively). At the protein level, IL-17 and RORgt significantly increased 8 and 30 days post BDL. In vitro, Stat-3p, NF-κB, Smad-2p and profibrogenic genes collagen I, III and TGF-b significantly increased in IL-17 heterodimer stimulated cultured HSC
Conclusions: Our results suggest that IL-17 (A and F) isoforms and their receptors are critical mediators of liver damage in the pathogenesis of early and advanced experimental cholestatic fibrosis. Th17 cells might represent an important source of IL-17. Heterodimeric IL-17 A/F potentially induces profibrogenic genes in HSC culture.

P0093

EFFECT OF MESENCHYMAL STEM CELL ON HEPATIC FIBROSIS IN THIOACETAMIDE-INDUCED CIRRHOTIC RAT MODEL

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Background and Aims: Cirrhosis is a long-term consequence of chronic hepatic injury with fibrosis and no effective therapy except liver transplantation is currently available for decompensated cirrhosis. However, some practical limitations in liver transplantation lead us to a need for new therapeutic paradigm in this field. Recent reports have shown that the mesenchymal stem cells (MSCs) have the plasticity to differentiate into some kinds of tissue cells and improve organ function. Hence, we investigated the effect of direct inoculation of human bone marrow derived MSCs (BM-MSCs) in thioacetamide (TAA)-induced cirrhosis in a rat model.
Methods: Adult Sprague-Dawley rats were allocated into three groups (each group, $n = 15$) as follows: G1, shame; G2, TAA-control; G3, TAA+BM-MSC. To induce cirrhosis, 200 mg/kg TAA injection was done twice a week for 12weeks in G2 and G3. 2×10^6 cells of amplified human BM-MSCs were injected directly into the right liver lobe twice, at weeks 6 and 8 in G3. At 12 weeks, the effect of BM-MSCs on cirrhosis was analyzed histomorphologically using Laennec scores. α-Smooth muscle actin (α-SMA) expression by immunohistochemical staining, relative expression of collagen type 1, and transforming growth factor β (TGF-β) were also evaluated by real-time reverse transcriptase-polymerase chain reaction.
Results: Laennec scores were 0, 5.4±0.7 and 3.7±1.06 in G1, G2 and G3, respectively. Histologically, BM-MSCs injected group (G3) showed significant suppression of hepatic fibrosis compared with TAA-control group (G2) ($P < 0.001$). Expressions of α-SMA (%) were significantly lower in G3 than in G2 (3.08±1.26 vs. 7.00±4.12, $P < 0.05$). Also, the relative expression of collagen type 1 and TGF-β1 in RT-PCR were 0.64±0.24, 2.06±0.51, 1.32±0.31 and 0.62±0.28, 5.89±3.05, 2.22±1.41 in G1, G2 and G3, respectively ($P < 0.005$).
Conclusions: Our results showed that BM-MSCs could attenuate liver fibrosis in rats with TAA-induced cirrhosis, raising the possibility for clinical use of BM-MSCs in the treatment of cirrhosis.

P0094

IMPAIRED 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2 ACTIVITY CONTRIBUTES TO RENAL SODIUM AVIDITY IN HUMAN CIRRHOSIS

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Background and Aims: The enzymes 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) mainly located in the liver and type 2 (11βHSD2) mainly located in the kidney and colon interconvert cortisol and cortisone. The balance of the enzyme activity decides the presence of the abundant cortisol with high affinity for the mineralocorticoid receptor (MR). The enzyme 11βHSD2 protects the MR in the kidneys from stimulation from cortisol, which has a higher affinity than aldosterone. Thus a down regulation of the 11βHSD2 in patients with cirrhosis is a potential pathophysiological mechanism in sodium retention in cirrhosis. We hypothesize that 11βHSD1/11βHSD2 balance is increased due to down regulation of 11βHSD2 activity in cirrhosis and related to renal sodium retention.
Methods: Thirteen patients with cirrhosis and 6 healthy controls were investigated. On sodium fixed diet analyses of cortisol/cortisone metabolites as well as the sodium excretion in the urine were performed. The metabolites of cortisol are 5β-tetrahydrocortisol (THF) and 5α-tetrahydrocortisol (a-THF) and the metabolite of cortisone is 5β-tetrahydrocortisone (THE). The balance of 11βHSD1 and 11βHSD2 activity is expressed as the ratio; THF + allo-THF/THE.
Results: The balance of 11βHSD1/11βHSD2 activity was found to be significantly increased in the patients compared with healthy controls; THF+allo-THF/THE (patients: 0.77±0.09; controls: 0.55±0.04, $p < 0.05$). The urinary sodium volume (UNaV) and the fractionated sodium excretion (FeNa) were significantly reduced in the patients; UNaV (patients: 15.7±5.5 mmol/day; controls 63.4±16.6 mmol/day, $p < 0.01$); FeNa (patients: 0.12±0.04; controls: 0.34±0.11, $p < 0.05$). Urine flow rate was at the same level in the two groups (patients: 1.05±0.11 ml/min; controls 1.33±0.09 ml/min, $p > 0.05$), whereas creatinine clearance was statistically significant reduced in the patients (patients: 76.6±5.6 ml/min; controls: 105.8±9.2 ml/min, $p < 0.05$).
Conclusions: The balance of 11βHSD1/11βHSD2 enzyme activity is increased in cirrhotic patients and it demonstrates a down

regulation of 11βHSD2 enzyme activity. This suggests that the protection of the mineralocorticoid receptor from cortisol activation by 11βHSD2 may be impaired. The sodium retaining mechanisms in the distal tubule in cirrhosis is not only mediated by aldosterone, but also by the activation of the mineralocorticoid receptor by cortisol.

P0095

ER STRESS ENHANCES LIVER FIBROSIS THROUGH MIR-150 DEGRADATION AND XBP-1 SPLICING

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Background and Aims: The unfolded protein response (UPR) is activated in several liver diseases, including NASH, cholestasis, viral hepatitis, alcoholic-induced liver injury and hepatocellular carcinoma. IRE1α, PERK and ATF6 are three parallel signaling pathways that mediate the UPR. IRE1α acts through both unconventional splicing of XBP1 mRNA and regulated IRE1-dependent decay of mRNA (RIDD), and also directly cleaves a subset of pre-miRNAs. The aim of our study is to define the mechanism by which the UPR enhances fibrogenesis and whether inhibition of the UPR could hinder development of cirrhosis.
Methods: Mice were injected with CCl_4 for 15 weeks and one group treated with 4μ8c, which is an inhibitor of IRE1α endonuclease activity. Fibrosis was evaluated by staining of liver sections with Sirius red and αSMA-antibodies. Stellate cells were isolated from healthy mice and stimulated with TGFβ +/− 4μ8c. miR150 levels were measured with qPCR. To show the direct effect of IRE1α RIDD activity on miRNA, an *in vitro* cleavage assay using pre-miRNA 5′-end labelled with ^{32}P-ATP was performed with the XBP1 stem loop as a positive control.
Results: Treatment of mice with 4μ8c significantly reduced fibrosis as shown by a reduction in both aSMA positive cells and Metavir score. This effects was likely due to activation of the UPR since 4μ8c treatment reduced the levels CHOP, BIP and spliced XBP1, which are markers of the activated UPR pathway. In support, *in vitro* studies using hepatic stellate cells isolated from mice and humans showed that 4μ8c inhibited TGFβ-induced differentiation into activated myofibroblasts. miR-150 is known to be anti-fibrotic and inibition of IRE1α with 4μ8c increased miR-150. Indirect evidence using various mutated IRE1α constructs and direct demonstration by recombinant enzyme cleavage showed that the RIDD activity of IRE1α destabilized and led to a downregulation of pre-miR-150 expression in activated myofibroblasts.
Conclusions: These data suggest that inhibiting IRE1α endonuclease activity attenuates liver fibrosis, possibly by affecting hepatic stellate cell differentiation via miR150 degradation.

P0096

D-DIMER AND FIBRINOLYTIC ACTIVITY IN PATIENTS WITH DECOMPENSATED LIVER CIRRHOSIS

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Background and Aims: Cirrhotic plasma could generate similar or even greater amounts of thrombin; a procoagulant imbalance has been introduced. Hyperfibrinolysis may complicate cirrhosis; standard tests (INR) fail to reflect bleeding tendency. Aim was to assess fibrinolytic system, clot lysis time and fibrinolysis of ascites.
Methods: We studied 33 pts with cirrhosis and 21 HS without no history of venous thromboembolism. EuroCLOT consortium was employed to study clot formation, morphology and fibrinolysis as dynamic process, measuring CLT (clot lysis time), CLR (clot lysis ratio), TAFI, PAI-1, t-PA, plasminogen, α2-antiplasmin, fibrinogen and D-dimer. We studied 11 A, 9 B, and 13 C cirrhotic pts. CP/MELD scores were significantly different among the three groups of pts. ($p < 0.001$). Systolic and diastolic blood pressure, heart rate, platelet counts, bilirubin and albumin levels were also different ($p < 0.001$).
Results: EuroCLOT data resulted significantly different (LagC, MaxabsC, CRC, Lys50t0, LysT, and LR) (from $p < 0.05$ to $p < 0.001$ CPB and CPC versus CPA and HS). Generated curves (AUC) were progressively decreased (from 585, to 316, 257, 1nd 129) ($p < 0.001$), so as TAFI values (from 8.4, to 8.32, 4.09, and 3.6 ug/mL, $p < 0.001$); PAI-1 and t-PA increased progressively, while α2-antiplasmin, plasminogen, and fibrinogen decreased progressively ($p < 0.001$). D-dimer plasma levels increased progressively and parallel to the severity of the disease. Ascites revealed small amounts of TAFI and increased levels of D-dimer ($p < 0.001$).
Conclusions: Liver cirrhosis is characterized by enhanced plasma fibrinolysis associated with the severity of the disease and this could be explained by the different methods used, the classical ones and the new global turbidimetric ones. Increased plasma and ascitic levels of D-dimer and altered fibrinolytic parameters were found in pts versus HS. Generated curves exploring both clotting and fibrinolytic parameters from EuroCLOT clearly showed delayed clot formation, decreased clot strength/hyperfibrinolysis, in parallel with the severity of the disease (Kleinegris MC et al, 2014). These data, expecially D-dimer levels both in plasma and ascites, further suggest that ascitic fluid is in continuous exchange with plasma, possibly through lympathic flux; moreover, absence of fibrinogen and increased fibrinogen/fibrin degradation products in this space suggests a possible role of ascites in the pathogenesis of coagulopathy of liver disease.

P0097

PRECISELY QUANTITATING THE DYNAMICS OF LIVER CIRRHOSIS IN A RAT MODEL

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Background and Aims: Detailed histologic changes in dynamic cirrhosis are yet to be fully available. The present study aims to address this issue using a structural quantitation tool for understanding the process and the potential impact on hepatic pathogenesis.
Methods: We used a CCl_4-induced cirrhotic rat model. Liver specimens ($n = 3–5$ per time point) were collected at 0, 6, 8 and 12 wks with treatment, and 2, 4, 8 wks after treatment, respectively. Samples' processing, histologic staging, and quantitative imaging analysis were performed routinely or as reported [J Hepatol 2014;61:260].
Results: To understand the process of cirrhosis initiation and progression, we measured the speed of changes ($mean_{T1}/mean_{T2}$/wk) in all structural fibrotic parameters between serial time points (Figure 1). At initiating stage (0–6 wk), the most significant changes happened to septal bridge; top 3 parameters are: crosslink number of septa collagen fiber (SCF) (67.44-fold, $p = 0.01$), long SCF number (65.04-fold, $p = 0.03$), and SCF width (60.54-fold, $p = 0.02$). At progressing stage (6–12 wk), the most significant changes mainly located in sinusoidal space; top 3 are: number of sinusoidal CF (FCF) (6.12-fold, $p = 0.03$), FCF crosslink (3.90-fold, $p = 0.00$) and long portal CF (3.12-fold, $p = 0.00$); from Ishak F5 to F6, significant increase happened in CF crosslink number (2.74-fold, $p = 0.01$), CF perimeter (2.53-fold, $p = 0.01$), and CF area (2.34-fold, $p = 0.02$). In the whole pathogenesis process (0–12 wk), the most dramatic change of scarring occurred to sinusoid, e.g. change in FCF length reached to 845.88-fold ($p = 0.00$).

During cirrhotic regression, at early 2 and 4 wks, most decrease happened to portal location; then it changed to septal bridge; fibrotic dissolving were faster at 2 wk than the rest time points. Even at the same stage of Metavir F4, there were significantly lower values of almost all fibrotic parameters of regressing cirrhosis in comparison with the ones of progression phases ($p < 0.05$).

Conclusions: In the present cirrhotic model, fibrosis grew faster at the initial stage, but slower down as cirrhosis progressing; at the progressing stage, the most dramatic fibrotic changes occurred to septa and sinusoid with the presence of increased crosslinking and long thick CF. Without injury, cirrhosis regressed fast within 2 wks, presenting a dynamic distribution. Regressing cirrhotic stage generally had lower level of fibrosis content than its progressing counterpart.

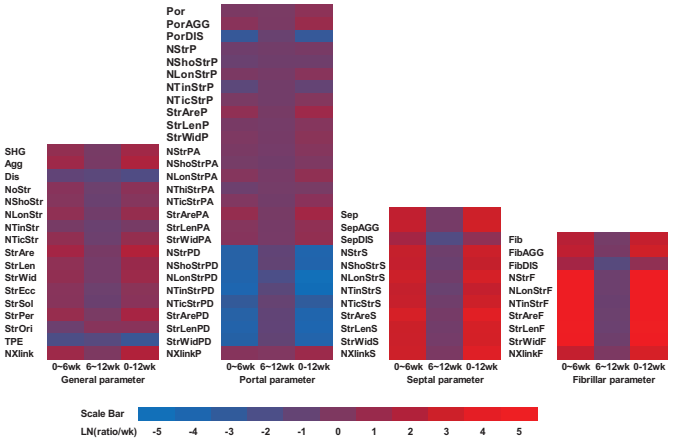

Figure 1. Velocity of changes in structural parameters in progressing cirrhosis.

P0098

MACROPHAGE INHIBITORY CYTOKINE-1 (MIC-1) IS ASSOCIATED WITH DEGREE OF LIVER FIBROSIS IN HCV-RELATED LIVER DISEASE – THE MILC STUDY

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Background and Aims: Macrophage Inhibitory Cytokine-1 (MIC-1/GDF15) is a divergent member of the TGF-β superfamily that is overexpressed (10–100 fold) in cancer and some chronic diseases. At these levels it has emerged as a potential mediator of anorexia/cachexia. In severe liver disease, nutritional state predicts morbidity and mortality and we hypothesised that MIC-1/GDF15 may also mediate these nutritional changes. The aim of this study was to assess the relationship of serum MIC-1/GDF15 to disease severity and nutritional status in HCV-related liver disease.

Methods: A cohort of HCV infected patients from St Vincent's Hospital, Sydney, Australia were stratified by degree of liver fibrosis using Fibroscan into three groups: no fibrosis (F0/F1; <7 kPa); moderate liver fibrosis (F2/F3; 7.1–13 kPa) and cirrhosis (F4; >13 kPa). Each participant undertook a range of tests, including: anthropometric assessments (BMI, Triceps Skin Fold and Mid-Arm Muscle Circumference); nutritional assessments (subjective global assessment, a 72-hour food diary, handgrip strength, International Physical Activity Questionnaire); metabolic assessments (REE and RQ by indirect calorimetry); and serological investigations, including serum MIC-1/GDF15 levels. The statistical relationship between these parameters and serum MIC-1/GDF15 levels were then examined.

Results: To date, 58 patients (mean age 57 years; males 72%) have been enrolled into the study: n = 18 (F0/F1); n = 18 (F2/3); and n = 23 (F4), including 4 decompensated cirrhotics. Serum MIC-1/GDF15 levels strongly correlated with Fibroscan scores ($r = 0.74$; $p < 0.0001$). There was a significant difference in mean MIC-1/GDF15 levels between the three groups (F0/F1, 873 pg/ml; F2/F3, 1205 pg/ml; and F4, 2042 pg/ml; KW $p \leq 0.01$). MIC1/GDF15 also correlated with lower albumin and increased AST, GGT and bilirubin ($p < 0.01$). No patient in the cohort had objective malnutrition, nor was there any significant difference in nutritional status between the 3 study groups. MIC1/GDF15 was not associated with any measures of nutrition or metabolism.

Conclusions: The data suggests a direct relationship between elevated serum MIC-1/GDF15 levels and the degree of liver fibrosis. No meaningful interpretation of the relationship between MIC-1/GDF15 and nutritional or metabolic parameters could be made because serum MIC-1/GDF15 serum levels were below anorexogenic levels, malnutrition was absent and nutritional status was no different across the study groups. This may reflect the lack of decompensated cirrhotics to date.

P0099

CHOLESTATIC LIVER FIBROSIS CORRELATES WITH INCREASED IL-17 AND TGFβ-2 EXPRESSION

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Background and Aims: IL-17, TGF-β1/2 are citokines involved in the development of kidney, pulmonar and liver fibrosis. However, their expression kinetics in the pathogenesis of cholestatic liver fibrosis has yet to be been fully explored. The aim of the study was to analyze the expression of IL-17, RORgt, NKp46, TGF-b1, and TGF-b2 in the liver of rats with bile duct ligation (BDL).

Methods: Male Wistar rats were sacrificed at days 8 and 30 after BDL. IL-17A, TGF-b1 and TGF-b2 expression was determined by qRT-PCR. IL-17, TGF-b1, TGF-b2, RORgt, and NKp46 were detected in liver homogenates by Western blotting. Collagen deposition was confirmed with Masson's trichrome stain.

Results: Hepatic IL-17A expression dramatically increased 350 fold and 10 fold, at 8 and 30 days post BDL, respectively. TGFb1 and TGFb2 expression significantly increased as well. At the protein level, IL-17, TGF-b1, and RORgt significantly increased 8 and 30 days after BDL. Interestingly, a significant increase in TGF-b2 and decrease of NKp46 was observed only 30 days after BDL. Unexpectedly, TGF-b2 exhibited stronger signals than TGF-b1 at the gene expression and protein levels. Histological analysis showed bile duct proliferation and collagen deposition.

Conclusions: Our results suggest that pro-fibrogenic cytokines IL-17, TGF-b1 and, strikingly, TGF-b2 are critical mediators of liver damage in the pathogenesis of early and advanced experimental cholestatic fibrosis. Th17 cells might represent an important source of IL-17, while NK cell depletion may account for the perpetuation of liver damage in the BDL model.

P0100
QT ADAPTATION DURING EXERCISE IN LIVER CIRRHOSIS: ADJUNCTIVE SIGNS OF CIRRHOTIC CARDIOMYOPATHY

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Background and Aims: QT-interval prolongation is frequently seen in liver cirrhosis. Whether it is simply a marker of disease severity is debated. Analysis of QT-interval behavior during physical exercise may disclose more specific abnormalities of cardiac repolarization of cirrhotic cardiomyopathy.
Methods: Thirty-eight outpatients with non-alcoholic liver cirrhosis and portal hypertension (32 males, aged 62±9 yrs) and 36 sex- and age-matched healthy subjects (32 males, aged 59±7 yrs) underwent bicycle exercise test with QT-interval measurement, echocardiographic and Doppler ultrasound analysis of systolic and diastolic left ventricular function, determinations of systemic hemodynamics and pro-brain natriuretic peptide concentration.
Results: Patients had longer Fridericia-corrected QT-interval than healthy subjects at baseline and peak-exercise and reduced chronotropic index, despite similar predicted workload. Corrected-QT shortening amount at peak-exercise was the same, however, in early-exercise corrected-QT increased in 6 healthy subjects and 25 patients, the increase being greater and significantly delayed in cirrhotic patients. QT hysteresis was greater in cirrhotic patients. Abnormal repolarization during exercise and recovery in patients with normal baseline corrected-QT did not correlate to Child–Pugh class score and hemodynamic alterations, whereas patients with >440 ms corrected-QT (n = 16) showed diastolic dysfunction and increased pro-brain natriuretic peptide plasma levels.
Conclusions: QT behavior during physical exercise supports the hypothesis of anomalous modulation of potassium currents in liver cirrhosis; only long rest corrected-QT correlates to clinical signs of cirrhotic cardiomyopathy.

P0101
SPECTRUM AND SHORT TERM OUTCOME OF RENAL IMPAIRMENT IN CIRRHOSIS

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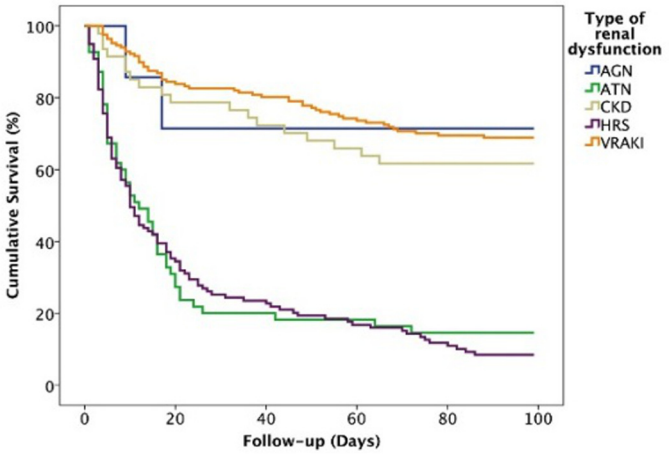
Background and Aims: Renal failure is the most powerful predictor of death in decompensated cirrhosis. We conducted this prospective, observational, cohort study to determine spectrum of acute kidney injury (AKI) as per IAC/ADQI, (International Ascites Club/Acute Dialysis Quality Initiative) definition in cirrhotics at admission or in hospital stay and study its outcome.
Methods: Cirrhotic patients diagnosed with AKI as per IAC/ADQI definition were enrolled and evaluated for the cause and type of AKI as per study definitions, investigated and treated as per study protocol. They were followed up to see reversibility of AKI and its effect on patients' in-hospital, 1 and 3 month outcome.
Results: 395 consecutive cirrhotics with renal impairment enrolled. In-hospital mortality 154 (39%). 226 (57.2%) survived at 1 month and 173 (43.8%) at 3 months. Median CTP 10 (5–14) and median MELD 25 (6–57). RRT (renal replacement therapy) required in 120 (30.4%). Infections were commonest precipitants with SBP, 84 (21.4%) the most common focus. VRAKI (volume responsive AKI) was commonest, 198 (50.1%). Among VNRAKI (volume non-responsive AKI), HRS (hepatorenal syndrome) seen in 85 (21.5%), ATN (acute tubular necrosis) in 55 (13.9%), and 9 (2.3%) had AGN (acute glomerulonephritis). Associated CKD (chronic kidney disease) seen in 48 (12.2%). VRAKI had best outcome followed by AGN and associated CKD. ATN/HRS had poor outcomes. Outcome was significantly poor in ACLF (acute on chronic liver failure), terlipressin non-responders and recurrent AKI. On multivariate analysis MELD score, serum albumin, presence of SBP and hypotension with inotropes requirement were significantly associated with HRS/ATN development. Presence of HE, low albumin and ATN/HRS were significantly associated with death.
Conclusions: AKI in cirrhotics was associated with significantly high in-hospital mortality, higher need for renal replacement therapy as well as progressively reduced 1 month and 3 month survival. VRAKI was the commonest with best outcome followed by HRS and ATN. Associated CKD had better 1 and 3 month survival than HRS/ATN. Presence of HRS/ATN, ACLF, recurrence of AKI in the same hospitalization were associated with poor 1 month and 3 month survival. MELD score, SBP, presence of hypotension with inotrope requirement were significant predictors of HRS/ATN development and presence of HRS/ATN, HE, low serum albumin were significant predictors of death. The survival in HRS/ATN did not correlate with infection-related parameters whereas that in VRAKI did.



P0102
DEGENERATION SIGNS OF PURKINJE NEURONS IN A NEW ANIMAL MODEL OF EPISODIC HEPATIC ENCEPHALOPATHY

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Background and Aims: Hepatic encephalopathy (HE) is a severe complication of cirrhosis. It has been classically considered as a reversible disease but several studies demonstrate that repeated episodes of HE may imply brain volume reduction and neuronal loss leading to persistent cognitive impairment. The aim of our study is the development of a new animal model of episodic HE, triggered by hyperammonemia and/or inflammation, which reproduces the features of HE patients and allows the identification of neurodegeneration evidences.
Methods: The animal model consists on the simulation of several episodes of HE in rats which previously underwent portacaval anastomosis surgery. The episodes were achieved trough the regular administration of ammonium acetate and/or lipopolisacharide (LPS). To confirm central nervous system damage, neurological status was assessed. After ten simulated episodes, brains were removed in order to quantify neuronal loss and to identify the signaling pathways involved in this process.

Results: Cognitive impairment was evaluated using a reflexes test, it revealed a lost of at least 50% of reflexes in 36% of the animals treated with ammonia; and an object recognition test, which evaluates memory and learning, shows a long-term mild decrease of those abilities in LPS-treated rats.
In cerebellum, neuronal counting proved a reduction in Purkinje cell population together with morphological changes attributed to degeneration process (shrinkage, dark cytoplasm and nucleus, and deformed nuclei) in all groups when compared with control.
Gene expression analysis evidenced a modulation of neuro-degeneration-related genes in cortex [adora2A Fold Change (FC): −2.45] and cerebellum (transtiretina FC: 17.45) but did not reveal any canonical pathway affected. The study of the pathways involved in neuronal death process in cerebellum shows an over-expression of senescence-related genes [IFN-γ FC = 2.15; cdkn1a (p21) FC: 1.88; cdkn2a ($p16^{INK4}$) FC = 1.68].
Conclusions: The neurological impairment found in patients is well reproduced by this new animal model of HE when ammonia is the precipitant factor of the episodes. Ammonia toxicity in central nervous system provokes gene expression changes in neurodegeneration-related genes, as well as Purkinje neurons death, probably through senescence pathways. The persistence of cognitive impairment after HE episodes resolution, observed in this animal model, could be caused by that premature senescence of Purkinje cells.

P0103
"PINRO" HYPOTHESIS FOR THE PATHOPHYSIOLOGICAL MECHANISM ABOUT HEPATITIS B VIRUS (HBV) RELATED ACUTE-ON-CHRONIC LIVER FAILURE (ACLF) PATIENTS – THE CRUCIAL ROLE OF SUBMASSIVE HEPATIC NECROSIS (SMHN)

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Background and Aims: "PIRO" is the current hypothesis for the mechanism of ACLF. After our group finding SMHN was the major pathological feature of HBV related ACLF patients, we put forward "PIN(necrosis)RO" is the mechanism for HBV cirrhotic patients.
Methods: 174 liver transplanted HBV cirrhotic patients with acute decompensation were enrolled. 40% (69/174) had histological evidence of SMHN. Clinical features about inflammatory response and multiple organ failure (MOF) were compared between SMHN positive and negative group whenever acute insults exiting or not. Microarrays of 33 cirrhotic livers were used to investigate gene expression changes in hepatic metabolism (first response) after SMHN.
Results: Acute insults were identified in 58.6% (102/174) enrolled patients. There was no difference in either inflammatory (white blood cells count, WCC) or MOF parameter (CLIF-ACLF) between insults identified and unidentified group ($p > 0.05$ for all). However, in acute insults identified group, SMHN positive patients (n = 44) had a significantly higher WCC ($7.2\pm3.9\times10^9$/L vs $4.3\pm3.6\times10^9$/L), CLIF-SOFA [8 (6, 10) vs 4 (2, 6)] and MELD [32 (25.3, 36.5) vs 13.5 (10, 20.5)] than SMHN negative patients (n = 58) ($p < 0.001$ for all). In the meantime, SMHN positive patients (n = 25) also had both significantly more severe inflammation (WCC, $7.22\pm3.84\times10^9$/L vs $3.75\pm1.61\times10^9$/L) and MOF [CLIF-SOFA, 8 (7.5, 10) vs 4 (4, 6)] than SMHN negative patients (n = 47) in acute insults unidentified group. Comparing to the SMHN negative cirrhotic liver, microarray data showed that 12 of 15 most down-regulated pathways in SMHN positive liver were related to the hepatic biosynthesis, catabolism and metabolism of different amino acids, fatty acids and glucose. That reflected the more severe hepatic function damaging in SMHN patients. 11 of 15 most up-regulated pathway were related to inflammatory molecules and immune response markers in SMHN positive liver, which reflected the more deranged immune response after SMHN.
Conclusions: Our data showed the necrosis (SMHN), rather than acute insults, determined the progression of first (the damage of hepatic metabolism) and second (inflammatory response) response as well as further development of MOF. Thus the pathophysiological mechanism underling ACLF would be "PINRO": predisposition → injury → (submassive hepatic) necrosis → deterioration of liver function (first response)/deranged systemic inflammatory response (second response) → multiple organ failure.

P0104
THE LIVER SINUSOID WITHIN A MICROFLUIDIC CHAMBER: A NEW TOOL FOR VASCULAR BIOLOGY RESEARCH

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Background and Aims: The liver sinusoid plays a key role in the development of liver diseases, including cirrhosis and portal hypertension. In addition, therapies to improve liver function

Table 1 (abstract P0103). Analysis of clinical features according to presence or absence of SMHN and acute insults

Characteristic	Acute insults identified			Acute insults unknown			Acute insults (+) vs (−)	
	SMHN(+) (N = 44)	SMHN(−) (N = 58)	*p*1	SMHN(+) (N = 25)	SMHN(−) (N = 47)	*p*2	SMHN (+), *p*3	SMHN (−), *p*4
Inflammatory parameters								
Leukocyte count ($\times10^9$/L)	7.22±3.87	4.30±3.63	<0.001	7.22±3.84	3.75±1.61	<0.0001	1.00	0.34
Neutrophil count ($\times10^9$/L)	5.03±3.02	2.67±3.14	<0.001	5.19±3.24	2.19±1.38	<0.0001	0.84	0.33
Multiple organ failures before LT								
CLIF-SOFA	8 (6, 10)	4 (2, 6)	<0.0001	8 (7.5, 10)	4 (4, 6)	<0.0001	0.220	0.310
SOFA	7 (6, 9)	5 (3, 7)	<0.0001	8 (6, 9.5)	5 (4, 7)	<0.0001	0.982	0.850
MELD score	32 (25.3, 36.5)	13.5 (10, 20.5)	<0.0001	34 (27, 36.5)	17 (13, 22)	<0.0001	0.332	0.091
Liver (TB ≥12.0 mg/dl)	35 (79.5%)	10 (17.2%)	<0.0001	21 (84%)	6 (12.8%)	<0.0001	0.649	0.526
Kidney (Cr ≥2.0 mg/dl)	6 (13.6%)	1 (1.7%)	0.041	3 (12%)	0 (0)	0.039	0.845	1.000
Cerebral (HE ≥ grade III)	5 (11.4%)	4 (6.9%)	0.494	6 (24%)	0 (0)	0.001	0.168	0.126
Coagulation (INR ≥2.5 or PLT ≤20×10^9/L)	18 (40.9%)	7 (12.1%)	0.001	18 (72%)	3 (6.4%)	<0.0001	0.013	0.506

*p*1: acute insults (+) SMHN (+) vs (−). *p*2: acute insults (−) SMHN (+) vs (−).
*p*3: SMHN (+) acute insults (+) vs (−). *p*4 :SMHN (−) acute insults (+) vs (−).

should also target sinusoidal cells.
However, there are no *in vitro* research tools that correctly mimic the unique features of the sinusoid: adequate spatial distribution of cells, biomechanical stimulation of the endothelial lineage and free paracrine interactions.
Our aim was to design, fabricate and validate a three-dimensional co-culture chamber with microfluidics that mimics the hepatic sinusoid.
Methods: A transparent PMMA chamber composed by 3 growing areas of 9.7 cm^2 arranged at different heights and separated by 0.5 mm was manufactured. The higher level integrated a microfluidic system allowing the application of homogeneous shear stress on a reinforced porous membrane where the endothelium is grown. The middle level had a second culture membrane, and on the lower level, the growing area was the base of the camera.
Functional validation: Endothelial cells stimulated with shear stress (5 dyn/cm^2), HSC, and hepatocytes were cultured for 24 h in the chamber. Morphology, viability and endothelial nitric oxide production was analyzed.
Translational Validation: The applicability of the chamber in the field of cirrhosis was assessed co-culturing activated HSC and capillarized LSEC under shear stress, or in static conditions. Furthermore, the effects of adding the vasoprotective agent simvastatin were analyzed and compared with traditional culture methods.
Results: *Functional validation:* Cells grown in the chamber showed excellent viability. Endothelial cells were aligned in the direction of shear stress, and increased their production of nitric oxide.
Translational Validation: Dysfunctional LSEC cultured in the device showed a marked improvement in their phenotype (activation of the KLF2 pathway and decrease in endothelin-1) that was not observed using ordinary culture methods. The shear stress-derived improvement in LSEC led to a beneficial paracrine effect on HSC (reduced collagen I and alpha-SMA). Simvastatin addition produced a strong protective effect on both cell types, which was significantly higher than that obtained using traditional "transwell" methods.
Conclusions: We herein describe a novel, versatile, easy to operate and highly reproducible device that can be applied in different fields of vascular biomedical research, including hepatology.

P0105
CHANGES IN THE CNS CONNEXIN 43 EXPRESSION AND FUNCTION IN THE PATHOGENESIS OF HEPATIC ENCEPHALOPATHY

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Background and Aims: Astrocytes, the most numerous glial cells, are hypothesized to play a role in the pathological mechanisms underlying hepatic encephalopathy (HE), although the clinical manifestations of HE are mainly neuronal. Astrocytes are extensively connected by gap junctions formed of connexins (Cxs), which may also exist as functional hemichannels allowing the exchange of molecules between the cytoplasm and the extracellular milieu. In pathological conditions, including HE, significant changes in astroglial anatomy and function may occur. In this study we investigated changes in the expression profile of Cx43 (predominant type of astroglial Cx) and hemichannel function in the cortex and cerebellum. The animal (rat) models of HE (bile duct ligation [BDL] and hyperammonemia) were used.
Methods: Cx43 mRNA and protein levels were quantified in the cortex and cerebellum of SHAM, BDL and hyperammonemic rats, using PCR and Western blots. Microelectrode biosensors were used for real time measurements of glutamate and lactate release by cortical and cerebellar slices prepared from the brains of SHAM, BDL and hyperammonemic animals.
Results: Cx43 mRNA expression was downregulated in the cortex and cerebellum of BDL and hyperammonemic rats. Western blots analysis revealed a reduction in Cx43 protein in the cortex. Biosensors showed a significant reduction in both tonic and stimulated lactate release in the cerebral cortex of hyperammonemic, but not BDL rats. Lactate release in the cerebellum of BDL and hyperammonemic rats was also decreased. Cortical slices obtained from BDL and hyperammonemic rats showed markedly higher glutamate release than that of SHAM animals under all conditions ($p < 0.05$). In the cerebellum, the tonic release of glutamate in hyperammonemic rats was higher than that of the SHAM animals. In both models a decrease in glutamate release due to hypoxia was observed.
Conclusions: The results of the present study suggest that HE is associated with significant changes in the connexin expression in the CNS. Altered Cx43 expression may play a role in a distinct neurochemical phenotype, as we report (lower lactate, higher glutamate) and is therefore hypothesised to play a significant role in the pathogenesis of the disease.

P0106
REDUCED EXPRESSION OF GLYOXALASE-I IN CIRRHOTIC LIVERS: A MECHANISM THAT COULD EXPLAIN HIGHER CONCENTRATION OF REACTIVE OXYGEN SPECIES (ROS) IN THE DEVELOPMENT OF CIRRHOSIS

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Background and Aims: Formation of ROS is an important mechanism in the pathogenesis of cirrhosis leading to release of proinflammatory cytokines and activation of NF-kB. ROS result, amongst others, from high concentrations of methylglyoxal (MGO) causing cytotoxicity. MGO is metabolized by glyoxalase-I (Glo-I). However, the expression and activity of Glo-I as catalytic enzyme in reduction of MGO during development of cirrhosis has not been elucidated so far. The aim of this study was to analyze Glo-I during the development of cirrhosis in hepatocytes (HEP), hepatic stellate (HSC) and sinusoidal endothelial cells (SEC).
Methods: Specific activity, protein- and mRNA-levels of Glo-I were measured via kinetics, western-blot, RT-PCR, immunohistochemistry and ELISA in liver cell-lines and primary liver cells (HEP, HSC, SEC) from normal and cirrhotic rats with early and advanced cirrhosis (CCl_4 treatment for 8 [8w] and 12 [12w] weeks, respectively). HSC were stimulated by LPS (100 ng/ml) for 24 hours in presence and absence of higher dosis of ethyl pyruvate (EP; 20 mM), that decreases MGO. Fibrosis (collagen-I, α-SMA) as well as inflammatory (TNF-α, NF-kB, p-ERK, nrf2) markers were measured to identify the anti-fibrotic and anti-inflammatory effects of EP.
Results: Expression of Glo-I was reduced in early and advanced cirrhosis in whole liver (8w: 34.3±4.2%; 12w: 18.4±2.1%; $p < 0.001$) as well as HEP (8w: 39.1±13.6%; 12w: 41.4±12.0%; $p = 0.018$), HSC (8w: 58.1±9.4%; 12w: 15.9±4.2%; $p = 0.032$) and SEC (8w: 51.2±9.0%; 12w: 16.8±5.8%; $p < 0.001$) compared to normal (100%). 24 h-treatment of HSC with EP led to concentration-dependent reduction of LPS-induced production of TNF-α (−/+ 20mM EP: 397.1±71.3 / 121.0±28.2 pg/ml, $p = 0.06$) as well as collagen-I (−/+ 20mM EP: 9.9±0.9 / 1.16±0.5 ng/ml, $p < 0.001$) and alpha-α (−/+ 20mM EP: 76.7±2.0 / 52.9±2.0 ng/ml, $p < 0.001$). Effects were mediated via reduction of NF-kB (compared to normal: LPS: 137.5±4.4; LPS+20mM EP: 84.4±2.6%; $p < 0.001$), p-ERK (compared to normal: LPS: 217.3±25.1;% LPS+20mM EP: 103.9±13.4%; $p < 0.01$) and stimulation of nrf2 (compared to normal: LPS: 69.1±3.2%; LPS+20mM EP: 168.1±8.1%; $p < 0.001$).

Conclusions: Glo-I expression is reduced in cirrhosis, including hepatic stellate cells, sinusoidal endothelial cells and hepatocytes. EP regulates MGO-levels by Glo-I in activated HSC leading to reduction of fibrosis and inflammation. Therefore, MGO by leading to higher amounts of ROS could play an important role in the development of cirrhosis.

P0107

REDUCED ABUNDANCE OF MINERALOCORTICOID RECEPTOR IN CIRRHOSIS: A MECHANISM THAT IS LINKED TO HYPOXIA AND PROINFLAMMATORIC CYTOKINES

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Background and Aims: Aldosterone induces fibrosis in liver through the mineralocorticoid receptor (MR). During development of cirrhosis the activation of renin-angiotensin-aldosterone-system results in higher levels of aldosterone. The aim of this study was to investigate the role of MR during the development of cirrhosis.
Methods: Male wistar rats were treated 5, 8 or 12 weeks (w) with CCl_4. Livers where excised and either conserved or treated for isolation of hepatocytes (pRH), hepatic stellate cell (pHSC) or endothelial cells (pLSEC). Gene expression was analyzed by realtime qRT-PCR. To gain more insight into the importance of MR in liver cirrhosis, HepG2 cells were treated with a combination of hypoxia and cytokines, mimicking conditions during development of cirrhosis, either with or without eplerenone (MR antagonist) or aldosterone (10 nM).
Results: In hepatocytes MR-mRNA was decreased after 12w CCl_4 treatment ($-\Delta\Delta$Ct: -1.46 ± 0.39 compared to control; $p<0.05$) compared to normal. In comparison, in pHSCs and pLSECs no alterations could be observed. Interestingly MR-mRNA content seemed to be higher in pRH (20-fold) compared to pHSC and pSEC in cirrhotic livers. To investigate if the alteration in MR expression leads to a change in MR dependent gene expression, serum and glycocorticoid regulated kinase (SGK-1) was analyzed. After 12w of CCl_4 treatment SGK-1 mRNA was reduced in whole liver lysate ($-\Delta\Delta$Ct: -3.88 ± 0.99 compared to control; $p<0.05$), pRH ($-\Delta\Delta$Ct: -1.26 ± 0.49 compared to control; $p<0.05$), but not in pHSCs ($-\Delta\Delta$Ct: 1.15 ± 1.07 compared to control; $p=0.40$) and pLSECs ($-\Delta\Delta$Ct: -0.77 ± 0.89 compared to control; $p=0.52$). To investigate the mechanism of MR regulation, HepG2 cells were incubated in mimicked cirrhotic milieu. While under normal conditions stimulation by aldosterone of the MR receptor was seen. MR protein expression decreases under combination of hypoxia and cytokine conditions regardless of presence of aldosterone or eplerenone.
Conclusions: In the liver the MR is mainly localized in hepatocytes. During cirrhosis the MR abundance is reduced. We propose that this is mediated by hypoxia and cytokines.

P0108

OVEREXPRESSION OF THE GENETIC EFFECTOR PATHWAY OF INFLAMMASOME IN PERIPHERAL BLOOD MONONUCLEATED CELLS (PBMC) OF PATIENTS WITH ACUTE ON CHRONIC LIVER FAILURE

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Background and Aims: Acute on chronic liver failure (ACLF) is a severe complication of cirrhosis characterized by organs failure and a high short-term mortality. Inflammation may be relevant in the pathophysiology of ACLF. Inflammasome is a multi-complex protein involved in the inflammatory immune response. We aimed to investigate the expression of genetic effector pathway of inflammasome in PBMCs of patients with ACLF and its prognostic relevance.
Methods: Seventy-two consecutive patients hospitalized for an acute decompensation of cirrhosis were included in the study and followed prospectively (group 1). Fifteen outpatients with uncomplicated cirrhosis were also included (group 2). Clinical, laboratory data and blood sample were collected at the admission in patients of group 1 and during a scheduled visit in those of group 2. Plasma levels of TNF-α, IL-6 were assessed by ELISA. Gene expression levels of NF-kB, Caspase-3, Caspase-1, TNF-α and IL-1β in peripheral blood mononucleated cells (PBMCs) was assessed by means of real time PCR.
Results: ACLF was diagnosed in 21 (29.2%) of patients in group 1. Patients with ACLF showed higher MELD score (26.5vs14; $p<0.001$) and CTP (11vs10; $p<0.05$). Plasma levels of TNF-α and IL-6 were found to be significantly higher patients in group 1 than in patients in group 2 ($p<0.05$ and $p<0.001$, respectively). In group 1, the plasma levels of TNF-α and IL-6 were found to be significantly higher in patients with ACLF than in those without ACLF (38.9vs20.2 pg/mL, $p<0.05$; 34.9vs15.4 pg/mL, $p<0.05$, respectively). Gene expression levels of NF-kB (35.26 vs 2.18; $p<0.03$), caspase-3 (29.55 vs 1.8; $p<0.03$), caspase-1 (75.57 vs 3.25; $p<0.05$), TNF-α (95.01 vs 2.8; $p<0.05$) e IL-1β (65.12 vs 18; $p<0.05$) were significantly higher in PBMCs from ACLF vs no-ACLF patients. In group 1, the mortality rate was significantly higher in patients with ACLF vs patients without ACLF (77.8 vs 37.5%; $p<0.004$). In group 1, gene expression levels of NF-kB (34.84 vs 2.18; $p<0.01$), Casp1 (69.56 vs 3.25; $p<0.03$), Casp3 (32.38 vs 1.89; $p<0.005$), TNFα (71.82 vs 2.81; $p<0.01$), IL-1β (63.66 vs 15.62; $p<0.03$) were higher in non survivors than in survivors.
Conclusions: Our data confirm that an excessive inflammation is involved in the pathophysiology of ACLF in patients admitted to hospital for an acute decompensation of cirrhosis. An overexpression of the genetic effector pathway of inflammasome in PBMC is a relevant mechanism of the excessive inflammation in patients with ACLF with a potential negative impact on survival.

P0109

ELEVATION OF CATHEPSIN L AND B EXPRESSION IN LIVER FIBROSIS: A STUDY IN MICE MODELS AND PATIENTS

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Background and Aims: Liver fibrosis and cirrhosis are characterized by excessive accumulation of extracellular matrix proteins as a result of wound healing response of liver to sustained injury. Extracellular matrix proteins deposited during liver fibrosis are well known substrates of cathepsin L (CTSL) and B (CTSB) which exhibits potent collagenolytic and elastinolytic activity. Present study was undertaken to investigate the expression of the potent ECM degrading proteases CTSL and CTSB in two different murine hepatofibrotic models and archival liver biopsy of patients.
Methods: In vivo hepatic fibrosis was induced by repeated intraperitoneal injections of CCl_4 for 10weeks in Swiss albino mice. Establishment of fibrosis was confirmed and expression of CTSL and CTSB as measured by qRT-PCR and immunohistochemistry (IHC). Liver tissues of phospholipid flippase ($Mdr2^{-/-}$) knockout mice at 4, 8 and16 weeks of age displaying the characteristics of primary biliary fibrosis were also included in the study to quantify the expression of CTSL and CTSB by qRT-PCR and (IHC). Further the expression of CTSL and CTSB by IHC was evaluated in patients with

severe liver cirrhosis and in patients with mild or no fibrosis along with biopsies of normal livers.
Results: CTSL was found to be significantly up regulated in CCL4 induced fibrotic mice (n = 5) at both RNA and protein level as compared to control mice olive oil treated (n = 6) while no significant change was evident in CTSB levels. The level of CTSL was found to be significantly upregulated by 1.5 and 1.8 fold in mdr2$^{-/-}$ as compared to wild type controls in 4 (n = 7) and 8 weeks (n = 8) old group of mice respectively whereas no changes was observed in 16 week old mdr2$^{-/-}$ mice as compared to the age matched wild type controls. Similarly CTSB expression was observed to be significantly higher in 4 week old mdr2$^{-/-}$ mice as compared to the wild type counterparts where as 8 and 16 old cohort of mice did not show any significant changes. Similar results were obtained when liver tissues from these groups of mice were stained for CTSL and CTSB. CTSL was found to be upregulated in liver samples of fibrotic patients (n = 10) as assessed by IHC and its expression increases with increasing grade of liver fibrosis. However, no significant changes in CTSB expression were observed in these samples.
Conclusions: CTSL expression increases with the extent of the severity of liver fibrosis in different murine models as well as in patient diagnosed with this pathology.

P0110
ACTIVATION OF MYOSTATIN AND MURF-1 IS INVOLVED IN MUSCLE WASTING IN MICE MODEL OF PRIMARY BILIARY CIRRHOSIS

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Background and Aims: Muscle wasting is a common feature in cirrhosis. Myostatin is well recognized as a negative regulator of muscle growth and differentiation. We therefore investigated if myostatin also contributes to muscle wasting in an experimental model of primary biliary cirrhosis (PBC).
Methods: 12-week old male wild-type (FVB) mice were used. PBC was induced by bile duct ligation (BDL). Control animals underwent bile duct exposure only (sham operated, SO). Five weeks after BDL, body weight was recorded and mice were sacrificed. Liver and spleen were removed and weighted. Liver histological analysis was performed to confirm PBC. Peripheral muscles were rapidly dissected at the sacrifice and weighed. Contractile properties of extensor digitorum longus (EDL) and soleus were studied in vitro in both isometric and isotonic conditions. Muscle samples were then rapidly stored for histological and biological analysis. mRNA expression for myostatin was evaluated together with components of the ubiquitin-proteasome system.
Results: Results refer to ten mice, 5 BDL and 5 SO. At the end of 5 weeks, a significant reduction in whole body weight of BDL mice respect to SO was observed. PBC was histological confirmed in all the BDL mice. Compared with SO controls, BDL mice had a reduction in skeletal muscle weights, mainly in quadriceps and gastrocnemius. A significant reduction in muscle cross sectional area was also observed. Muscle wasting was also associated with a reduction in maximum specific force and with a decrease in the resistance to fatigue in EDL muscles. A significant increase in myostatin expression in BDL mice with respect to SO was observed. Because previous studies on cachectic experimental animal models suggest that myostatin induces muscle atrophy by activating the expression of components of the ubiquitin-proteasome, we have also investigated the expression of Atrogin-1 and MuRF-1. An upregulation of MuRF-1 expression was confirmed in BDL mice with respect to SO control, while atrogin expression was similar in the two groups.
Conclusions: Muscle wasting is a characteristic complication in experimental models of PBC. Loss of skeletal muscle mass is associated with a reduction of muscle contractile capability, with a more selective involvement of fast-twitch fibers. The increased expression of myostatin and MuRF-1 suggested that a reduction in protein synthesis together with the activation of ubiquitin pathway are responsible for muscle wasting in this model of liver cirrhosis.

P0111
WHAT MIGHT BE THE ROLE OF DUCTULAR REACTION IN LIVER CIRRHOSIS?

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Background and Aims: The ductular reaction is a standard component of liver cirrhosis. However, its role in the process is debated. The close relationship between the ductular cells and myofibroblasts suggests a function for them in the process of fibrogenesis. It has been also proposed that the decreasing mitotic activity of the senescent hepatocytes is balanced or replaced by the proliferation and differentiation of ductular cells. We have addressed these issues in human cirrhotic livers and studied the dynamics of hepatic fibrosis in two well-known experimental models.
Methods: Fifty-six explanted human cirrhotic livers were analyzed by morphometry. The extent of fibrosis was characterized by Picro-Sirius red staining, smooth muscle actin (SMA), cytokeratin 7 (CK7) and Ki-67 immunohistochemical staining was also performed on representative histological sections.
Hepatic fibrosis was induced in male C57Bl/6 mice by thioacetamide (TAA; 300 mg/l in drinking water) and phenobarbital/carbon tetrachloride (PhB/CCl_4; 0.5 g/l in drinking water/0.2 ml/kg twice a week). The animals were sacrificed after 6, 9, 12, 15 and 18 weeks of treatment. The same morphometric parameters were studied as on the human samples (except SMA), and the proliferative activity was characterized by bromodeoxyuridine (BrdU) incorporation.
Results: In the human samples the Picro-Sirius stained fibrotic area correlated positively with the thickness of fibrous bundles, and the extent of SMA (myofibroblast) positivity. The extent of SMA staining increased parallelly with the CK7 positive ductular reaction. We found low proliferative activity in hepatocytes and ductular cells in all of our samples (in most cases below 1%). The proliferative activity of the hepatocytes correlated positively with the Ki-67 labeling of the ductular cells but inversely with the septum thickness. Both experimental animal models were characterized by slowly progressing fibrosis, with low but mostly constant level of hepatocyte proliferation. The extent of ductular reaction slowly increased, however the proliferative activity of the ductular cells decreased with time in the TAA model. Neither of these two parameters changed significantly in the PhB/CCl_4 model.
Conclusions: Both our human and experimental data support the potential connection among ductular reaction, myofibroblast activity and fibrosis, but contradict the proposed compensatory growth function of the ductular cells.

P0112

CYTOPATHOLOGICAL CHANGES ASSOCIATED WITH HEPATOCYTE SENESCENCE DURING EVOLUTION OF CIRRHOSIS AND MONITORING SENESCENCE IN CELL CULTURE SYSTEM (Huh7 CELLS)

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Background and Aims: Cellular senescence is a permanent growth arrest condition involved in process of ageing and tumor suppression. Identification of senescent cells is challenging particularly in primary tissues. Purpose of this study was to evaluate cytopathological features associated with senescence in conditions of cirrhosis and following doxorubicin treatment in hepatocellular carcinoma cell line (Huh7).
Methods: Huh7 cells were treated with low dose of doxorubicin (2μM, 2 hr) to induce premature senescence, which was evaluated by morphology (bright field microscopy and electron microscopy), SA-β-galactosidase staining and p21 expression. Liver sections from pre-cirrhotic (Metavir stage3) and cirrhotic conditions (Metavir stage4) were evaluated for presence of senescent like cells by combination of p21 expression together with cytomorphological feature of nuclear enlargement and vacuolation.
Results: Doxorubicin treated Huh7 cells showed enlarged cytomorphology, multinucleation, and positivity for SA-β-galactosidase activity coupled with G2/M phase cell cycle arrest indicative of premature senescence. Huh7 senescent cells showed accumulation of autophagic vacuoles, prominent lysosomes and mitochondrial-endoplasmic reticulum coupling. Compared to pre-cirrhotic condition, the hepatocytes in cirrhotic liver showed higher percentage of p21 nuclear immunoreactivity with prominent nuclear vacuolation (Precirrhotic 2.74+ 0.33, Cirrhotic 7.35+1.6; $P < 0.006$) and increased nuclear volume (Precirrhotic 147.46+9.28, Cirrhotic 202.32+10.2, $P < 0.0005$). In cirrhosis, p21 expression was more around periseptal region whereas in precirrhosis, it appeared uniform throughout the lobule.
Conclusions: Premature senescence in both in vitro and in vivo conditions showed cytomorphological changes indicative of adaptive response of senescent cells. Additionally increased p21 expression coupled to nuclear vacuolation appears as a good marker to identify senescent cells in primary tissues.

P0113

REGIONAL CEREBRAL WATER CONTENT IN HEPATIC ENCEPHALOPATHY MEASURED BY MRI

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Background and Aims: The pathophysiology of hepatic encephalopathy (HE) may involve cellular cerebral edema due to osmotic swelling caused by glutamine accumulation. However, this has only been studied in patients with covert HE or by indirect methods like diffusion tensor imaging (DTI). DTI parameters only give information about water molecule diffusion whereas quantitative estimation of absolute brain tissue water content by magnetic resonance imaging (MRI) offers precise characterization of the micro-structural changes.
Methods: We measured the absolute brain water contents and DTI parameters with anatomical resolution by MRI in 8 cirrhosis patients during an episode of overt HE type C. Six patients with cirrhosis and no history of HE and 12 healthy age matched control subjects were also scanned. Three of the HE patients were rescanned after recovery from HE. The images were normalized to the Montreal Neurological Institute Standard Brain and volumes of interest from a probabilistic brain atlas were used for image analysis.
Results: Temporal lobe water was 86±0.03%, 85±0.04%, and 82±0.04% in HE, cirrhosis, and controls, respectively. The corresponding cerebellum water was 86±0.02%, 84±0.03%, and 82±0.02%. The frontal lobe water was 84±0.03% in both HE and cirrhosis patients and 82±0.02% in the controls ($P < 0.05$, all regions HE vs. controls). The whole brain water contents of the three patients with overt HE fell after recovery by 1±0.02%. DTI parameters revealed changes in the white matter that correlated with the absolute water content.
Conclusions: Our data quantitatively demonstrate low-grade cerebral edema in several brain regions in patients with overt HE and suggest that the brain edema may be reversible with recovery from HE. Furthermore our data support that changes in DTI parameters can be used as an indirect measure of alterations in the absolute water content.

P0114

CIRRHOSIS IS ASSOCIATED WITH AVASCULAR NECROSIS, BUT NOT OSTEOARTHRITIS – A DANISH POPULATION-BASED STUDY

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Background and Aims: Cirrhosis causes systemic inflammation, but it is unclear whether it increases the risk of joint disease. We aimed to examined the association between cirrhosis and two joint diseases: osteoarthritis of the hip or knee, and avascular necrosis.
Methods: We used the Danish National Patient Registry to identify all Danish residents diagnosed with cirrhosis in 1994–2011 and matched them 1:5 by age and gender to non-cirrhotic reference individuals from the general population. We excluded persons with a previous diagnosis for osteoarthritis, avascular necrosis, and fractures of the hip, distal femur, patella or proximal tibia. We used stratified Cox regression to compute the hazard ratios (HR) for osteoarthritis and avascular necrosis for cirrhosis patients vs. reference individuals adjusted for comorbidity including alcohol related disease. We used the cumulative incidence function with death as competing risk to compute the 5-year risk of osteoarthritis and avascular necrosis.
Results: We identified 52,752 patients with cirrhosis and 263,760 reference individuals. The median age was 57 years, and 65% were men. We excluded 40% of cirrhosis patients and reference individuals because they had a history of an outcome or a relevant fracture. The HR for osteoarthritis in cirrhosis patients compared to reference individuals was 0.94 (95% CI: 0.54–1.66). Cirrhosis patients' HR for avascular necrosis was 8.42 (95% CI: 2.44–29.1). The 5-year risk of developing osteoarthritis was highest for reference individuals (3.3% vs. 2.2%) and both groups' 5-year risk of avascular necrosis was less than 0.1%.
Conclusions: Cirrhosis is unrelated to osteoarthritis, but is a strong risk factor for avascular necrosis, although it remains a rare condition.

P0115

SERUM BILE ACIDS IN CIRRHOSIS DEPEND ON AETIOLOGY

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Background and Aims: Elevated serum levels of bile acids are suggested to be diagnostic tools as well as predictors for mortality. Bile acids are metabolized by gut bacteria and therefore their composition depends on microbiome alterations. Furthermore bile acids can modulate gut permeability. Both microbiome and gut permeability are likely to differ between aetiologies. Therefore we hypothesize that bile acid concentrations in serum of various forms of cirrhosis are different form each other.
Methods: Bile acid profiles (cholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid, ursodeoxycholic acid) of 101 cirrhotic patients (56 alcoholic, 20 chronic hepatitis C, 25 others) and 10 healthy controls were determined as unconjugated acids and as taurine and glycine conjugates using a tandem mass spectrometry method. Gut permeability marker diamine oxidase and sCD14 were measured by ELISA, sucrose recovery and lactulose-mannitol-ratio by differential sugar absorption test (HPLC).
Results: When compared to healthy controls all cirrhotic groups show a higher abundance of conjugated bile acids in the serum which is particularly high in alcoholic cirrhosis. Patients with hepatitis C induced cirrhosis show normal values of unconjungated bile acids, while alcoholic and other aetiologies have significantly increased levels. Alcoholic cirrhosis is associated with the steepest increase in serum bile acids, with average values approximately 20 times higher than in healthy controls and 2–3 times higher than in other types of cirrhosis. Gut permeability is also elevated in all cirrhosis groups and highest in alcoholic cirrhosis. Nevertheless no clear association between serum bile acids and permeability marker could be found. Also ursodeoxycholic acid levels in serum were not associated with gut permeability.
Conclusions: Serum bile acid concentration and composition is strongly dependent on aetiology of cirrhosis. A possible explanation could be that the microbiome is subjected to different modulators like alcohol consumption, low bowel motility, antibiotic treatments etc. which might vary considerably between aetiologies. A closer look into the microbiome of cirrhotic patients could bring valuable answers. Associations between gut permeability were weak and their clinical relevance is questionable.

P0117

INFECTION IN HOSPITALIZED CIRRHOTIC PATIENTS AND DIABETES MELLITUS: IS THERE A LINK?

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Background and Aims: Infection is a frequent complication in patients with chronic hepatic disease (CHD) which contribute to a significant morbidity and mortality. Diabetes mellitus (DM) is a predictive factor for infection and its prevalence is higher in patients with CHD. Aims: To evaluate the prevalence of DM in patients with CHD and determinate the association between DM and infection.
Methods: Retrospective analysis of the clinic data of hospitalized patients with CHD admitted in a gastroenterology ward. Patients were divided in two groups: A-with infection, B-without infection. Basic demographic data, past medical history, clinic and laboratory data, type of infection, severity of liver disease and mortality were compared in both groups. Excluded: incomplete data, patients with neoplasm or immunosuppressed, hospitalization or use of antibiotics/immunosuppressive drugs in the last 3 months, gastrointestinal bleeding, institutionalized patients.
Results: 100 patients were included (A-55, B-45), 81.6% were male and mean age was 61.2±11.4 years. DM prevalence was 33% (A-23, B-10), significantly higher in group A (41.8% vs 22.2%). Main causes of infection: spontaneous bacterial peritonitis (34.5%), urinary tract infection (29%) and respiratory infection (25.5%). There was no statistically significant difference in the cause of infection between both groups ($p > 0.05$). Demographic data, etiology and severity of liver disease were similar in both groups ($p > 0.05$) and between diabetic and non-diabetic patients ($p < 0.05$). Univariate analysis of laboratory parameters shows significant difference ($p < 0.05$) in both groups (A-B) regarding fasting blood glucose (187±37.4 vs 95.8±9.2 mg/dL) serum albumin (2.9±0.4 vs 3.3±0.68 mg/dL), leukocytes (11.7±2.3 vs 5.4±1.8×10^9/L) and c-reactive protein levels (5.2±3.2 vs 1.9±2.4 mg/dL). The number of hospitalization days (18.2±4.2 vs 9.8±3.8 days) and mortality rate (27.3% vs 4.4%) was higher in group A ($p < 0.05$), without difference between diabetic and non-diabetic patients ($p > 0.05$).
Conclusions: This study concludes that there is a higher prevalence of diabetes mellitus in hospitalized patients with CHD, mainly those with infection.

Cirrhosis and its complications: b. Clinical aspects

P0118

PHASE 1–2 CLINICAL TRIAL IN PATIENTS WITH DECOMPENSATED LIVER CIRRHOSIS TREATED WITH BONE-MARROW DERIVED ENDOTHELIAL PROGENITOR CELLS

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Background and Aims: Bone marrow-derived endothelial progenitor cells (EPC) seem to promote liver regeneration and improve survival in animal models of acute and chronic liver disease.
The aim of this clinical trial was to evaluate the safety, the efficacy and the feasibility of the treatment with autologous EPC in decompensated liver cirrhosis and to analyse the relation between the characteristics of the cellular product and the effect on liver funtion and portal pressure.
Methods: Patients with Child–Pugh ≥8 liver cirrhosis were included. Bone marrow (BM) blood (50 ml) was withdrawn for *ex vivo* differentiation and selection of EPC. The final product was resuspended in 50 ml and injected via hepatic artery. Patients underwent clinical, biochemical and radiological follow-up for 12 months. Surface markers (CD133, CD34, CD117, VEGFR-2 and-1, CD31, vWF) and functional activity of EPC (acLDL-uptake and Ulex-lectin binding) were analysed by flow cytometry. HGF, VEGF, IGF-1, IL-6, EGF, SDF-1 and CT-1 were determined by ELISA in supernatant before administration.
Results: Twelve out the 14 patients included underwent BM harvest. EPCs were generated from BM mononuclear cells and 11 patients were treated (global feasibility 91%). Three patients died

at day 25, 85 and 166. No treatment-related severe adverse events after a median follow-up of 182 days (range 27–364) were observed. A significant improvement of MELD score (p 0.042) and INR (p 0.012) was observed in the first 90 days after treatment. Considering the 9 patients alive at 90 days, hepatic venous portal pressure (HVPG) improved in 5 patients (55.5%), remained stable in 2 (22.2%) and worsened in 2 patients (22.5%). Phenotype analysis showed that patients with an improvement of MELD score received an higher amount of cells expressing VEGFR-2 (13.8% vs 23.2%;p 0.024), vWF (72% vs 98%;p 0.048) and Ac-LDL uptake ability (21.7% vs 72.1%;p 0.048) compared to patients who did not improved. Moreover, the cells infused in patients showing MELD score improvement tended to produce higher amounts of IGF-1 and HGF

Conclusions: The treatment with BM-EPCs in patients with decompensated cirrhosis is safe an feasible and might have therapeutic potential. Patients treated with a cellular solution with an higher amount of functionally active EPC showed an improvement of liver function suggesting that these cells could be useful for the treatment of liver cirrhosis. A refinement in the process of cell selection may be critical in order to enhance the therapeutic effect.

P0119

VALIDATION OF THE NACSELD INFECTION-RELATED ACUTE-ON-CHRONIC LIVER FAILURE (I-ACLF) SCORE IN AN INDEPENDENT MULTICENTER COHORT

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Background and Aims: The infection-related acute-on-chronic liver failure (I-ACLF; ≥2 extra-hepatic organ failures) assessment is based on extra-hepatic organ failures, and was developed in a prior multicenter prospectively enrolled cohort of infected cirrhotic patients (NACSELD North American Consortium for the Study of End-Stage Liver Disease, Hepatology 2014). Assessment of extra-hepatic organ failures has been proposed as a simple bedside tool to assess mortality risk in admitted infected cirrhotic patients. *Aim:* To validate the accuracy of I-ACLF's ability to predict 30-day mortality (defined as in-hospital death or discharge to hospice) in a separate multicenter prospectively enrolled cohort of infected cirrhotic patients.
Methods: We utilized another cohort in the NACSELD database of tertiary care hepatology centers that prospectively enroll admitted cirrhotic patients (n = 816). We selected patients with an infection (n = 336) for this comparison with the original cohort. Organ failures assessed were: (1) shock, (2) encephalopathy (West-haven grade III/IV; HE), (3) renal (need for dialysis), and (4) respiratory (mechanical ventilation).
Results: Infected cirrhotic patients were most commonly Caucasian (79%) men (61%) with a mean age of 57 who had alcohol-induced cirrhosis (43%). Admission lab values were: creatinine 1.43 mg/dL, serum albumin 2.64 mg/dL, and median MELD and Child score were 19, and 10 respectively. Similar to the prior experience, we found that 30-day survival decreased with increasing numbers of organ failures; survival was 95% in patients without organ failure, 68% in those with 1, 46% in those with 2, and 33% in those with 3 organ failures (Table 1). The greatest decrement in survival occurred when patients met criteria for I-ACLF. Multivariable modeling documented that I-ACLF was the strongest predictor of death (OR of survival = 0.10, CI 0.04–0.25) after controlling for admission MELD (OR = 0.93, CI 0.88–0.98), WBC (OR = 0.71, CI 0.42–1.21), serum albumin (OR = 1.06, CI 0.55–2.04), and second infection (OR = 1.30, CI 0.47–3.56).

Table 1

	30-day survival		
Organ failure	Without organ failure	With organ failure	p-value
HE	91%	64%	<0.0001
Renal	91%	43%	<0.0001
Shock	93%	50%	<0.0001
Renal	91%	43%	<0.0001
Any One	95%	68%	<0.0001
I-ACLF (Extra-hepatic organ failures)			
Any two	94%	46%	<0.0001
Any three	91%	33%	<0.0001

Conclusions: I-ACLF has been independently validated in a separate large multicenter prospective trial as a simple bed-side tool to predict 30-day mortality in admitted infected cirrhotic patients.

P0120

THE 3-MONTH READMISSION RATE REMAINS UNACCEPTABLY HIGH IN A LARGE MULTI-CENTER COHORT OF CIRRHOTIC PATIENTS

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Background and Aims: Readmissions after discharge are an important indicator of care quality. However, factors associated with readmissions in cirrhosis are unclear from a multi-center perspective. *Aim:* To evaluate determinants of 3-month readmissions in a multi-center cohort of hospitalized cirrhotics.
Methods: NACSELD (North American Consortium for the Study of End-stage Liver Disease) is a multi-center database of liver centers that enroll inpatient cirrhotics. Data collected include reasons for index (when pt was enrolled) and re-admissions, medications (PPI, rifaximin, lactulose, SBP prophylaxis), cirrhosis severity at admission/discharge days. Pts discharged home were followed for 3 mths for readmissions, transplant and death. Readmission determinants were analyzed using logistic regression.
Results: 578 patients were enrolled. During index hospitalization, 77 died/went to hospice and 20 were transplanted. Thus, 481 pts (age 56 yr, 55% men, 37% HCV, 30% infection as admission reason) were analyzed. *3 month outcomes:* 77% were alive without transplant, 11% were transplanted, 12% died. *Readmissions:* 50% of patients were readmitted at least once; 56% had 1, 28% had 2, and 16% had ≥3 readmissions. These rates were similar between those admitted with/without infection (50% vs 51%, p = 0.8) during the index hospitalization. Most readmissions were infections (40%) followed by liver-related complications (17% anasarca, 11% GI bleed, 9% TIPS, 7% AKI, 5% HE) and 11% others.
Two regression models were created; *Using index hospitalization day of admission values:* A high MELD score (OR 1.1, p = 0.001),

lactulose use (corollary for HE, OR 1.6, p = 0.05) and male gender (OR 1.6, p = 0.05) predicted readmission while high serum albumin was protective (OR 0.57, p = 0.004). Infection as the index admission reason, diabetes or WBC were not predictive. *Using index hospitalization discharge values:* The only predictors of 3-month readmissions on discharge were MELD (OR 1.1, p = 0.002) and SBP prophylaxis (OR 1.95, p = 0.05). Nosocomial infections, diabetes, infections on admission and other medications were not predictive.
Conclusions: Readmissions occurred in 50% of hospitalized cirrhotics within 3 months of discharge, regardless of infections being the reason for the index admission, in this prospective multi-center study. Without intervention, these pateints likely be readmitted, most commonly for infections. Strategies to prevent readmissions are urgently needed, especially in higher MELD patients with HE and lower serum albumin who are on SBP prophylaxis.

P0121
ANGIOTENSIN II INHIBITION TO TREAT SEVERE LIVER FIBROSIS: A DOUBLE-BLIND RANDOMIZED TRIAL (ANRS HC19)

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Background and Aims: Cirrhosis prevention is a major target in chronic liver disease treatment. Angiotensin II type 1 receptor antagonists (ARA2) have shown anti-fibrotic properties in numerous pre-clinical and clinical studies. We thus evaluated ARA2 administration in a large patient population.
Methods: 166 patients with chronic hepatitis C (CHC) and Metavir F stages 2 or 3 were allocated to receive either irbesartan (I) 150 mg/d or a placebo (P) per os for 2 years in 27 centers. All patients had contraindications for or refused IFN-based regimens. The patients had clinical evaluation, liver biopsy, and non-invasive fibrosis tests (blood and stiffness) at inclusion and end of follow-up. Liver biopsies were centrally evaluated with Metavir staging by expert consensus and detailed automated morphometric measures including 44 descriptors, among which was porto-septal fibrosis area (main judgment criteria, Sandrini Modern Pathol 2014), to obtain morphometric scores for significant fibrosis (SF) and cirrhosis (F4).
Results: Baseline characteristics were: 58% male, age 56±9 yrs. Treatment groups were well balanced except for Metavir F at central reading, P vs I respectively, F1: 4.9 vs 1.2%, F2: 75.6 vs 63.1%, F3: 17.1 vs 33.3%, F4: 2.4 vs 2.4% (p = 0.048); this was also suggested by morphometric F4 score: 0.13±0.30 vs 0.16±0.31, p = 0.07. Paired liver biopsies were available in 79% of patients but analyzed in ITT. Changes in morphometry were, P vs I respectively: porto-septal fibrosis area: 0.43±2.19 vs 0.26±2.40%, p = 0.73; morphometric SF score: 0.02±0.28 vs 0.02±0.25, p = 0.75; morphometric F4 score: 0.08±0.36 vs 0.07±0.36, p = 0.20. There was an interaction (p = 0.002) between treatment and fibrosis stage in F4 score with opposite treatment effects between F1+F2 (0.12±0.29 vs 0.03±0.25, p = 0.04) and F3+F4 (−0.07±0.55 vs 0.15±0.49, p = 0.24). The rates of Metavir progression/regression (≥1F/≤1F) were, P vs I respectively: 12.1 vs 16.9% / 16.7 vs 14.8%, p = 0.84. Changes in liver blood fibrosis tests and stiffness were not significantly different between both treatment groups. No serious adverse events were attributed to treatment. Mean arterial pressure changes were: −3.5±11.3 vs −9.3±17.0 mmHg, p = 0.06.
Conclusions: A 2-year regimen of Irbesartan 150 mg/d did not significantly alter the course of liver fibrosis pathological patterns in CHC, even as assessed by precise morphometry. However, anti-fibrotic effect might depend on baseline fibrosis level, as already suggested.

P0122
TYPE 2 DIABETES REDUCES OVERALL SURVIVAL AND INCREASES THE RISK OF HEPATIC DECOMPENSATION IN HCV-RELATED LIVER CIRRHOSIS. RESULTS FROM A PROSPECTIVE LONG-TERM STUDY DURING 8 YEARS

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Background and Aims: Type 2 diabetes mellitus (DM) frequently coexists with liver cirrhosis, and may be associated with an increased risk of complications and mortality. We examined the influence of DM/insulin resistance (IR) on overall survival and liver-related complications.
Methods: This is a prospective inception cohort study of 250 subjects with compensated HCV-related cirrhosis followed during 416 weeks. Patients with HCV who were non-responders to, ineligible for and intolerant of peginterferon plus ribavirin were included. Significant prognostic indicators for overall survival or presence of a first event of hepatic decompensation were determined using univariable and multivariable Cox regression.
Results: At baseline, 67 patients (27%) had DM. During the follow-up, 28 deaths (6 nonliver- and 22 liver-related deaths or transplant) and 55 first events of decompensation occurred. After adjustment for covariates, the overall survival was significantly lower in diabetic patients (74%; 95% CI: 67–82) than those without diabetes (88%; 95% CI: 83–92), P = 0.04. Similarly, the occurrence of a first event of hepatic decompensation was higher in presence of diabetes (64%, 95% CI: 59–70) as compared with nondiabetic patients (41%, 95% CI: 33–47), P = 0.01. Additionally, subjects with a HOMA-IR >5 showed lower rates of survival (76%, 95% CI: 67–82) than those with HOMA-IR between 3 and 5 (88%, 95% CI: 84–94), and <3 (96%, 95% CI: 91–99), P = 0.04. The presence of hepatic decompensation were higher in patients with HOMA-IR between 3 and 5 (58%, 95% CI: 52–65) and >5 (66%, 95% CI: 61–74) than those with HOMA-IR <3 (34%, 95% CI: 30–41), P < 0.001; however, no differences were found between subjects with HOMA-IR ranging between 3 and 5 and HOMA-IR >5. Cox regression analysis revealed that presence of varices (HR = 2.5), male sex (HR = 2.4), DM (HR = 1.9), platelets count (HR = 0.98), HOMA-IR >5 (HR = 1.9) and TGO/TGP ratio (HR = 2.6) were independently associated to overall mortality. In addition, presence of varices (HR = 2.9), DM (HR = 2.1), mean arterial pressure (HR = 0.95), platelets (HR = 0.98) and HOMA-IR >3 (HR = 1.98) were predictors of hepatic decompensation.
Conclusions: Our study showed that presence of DM might reduce the overall survival and increase the risk of hepatic decompensation in compensated HCV-related cirrhotic patients. Likewise, a marked IR is associated to reduced survival and increased risk of hepatic decompensation.

P0123
IMPACT OF SHUNT NEGATIVE HYPOXEMIA ON SURVIVAL IN LIVER TRANSPLANT CANDIDATES

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Background and Aims: Hepatopulmonary syndrome [HPS] results when intrapulmonary shunting causes hypoxemia in cirrhosis. It occurs in up to 30% cirrhotics and increases mortality. However, hypoxemia has also been observed in the absence of intrapulmonary shunting and without evidence of intrinsic lung disease in cirrhosis, but is not well characterized. We studied shunt negative hypoxemic (SNH) cirrhotics relative to control and HPS patients in a multicenter cohort of liver transplant evaluants.
Methods: Patients undergoing LT evaluation at 7 US centers (Pulmonary Vascular Complications of Liver disease Group) underwent contrast echocardiography (CE), arterial blood gases, pulmonary function tests (PFT) and chest imaging. Those with moderate to severe PFT abnormalities, portopulmonary hypertension, or intracardiac shunting were excluded. HPS was diagnosed by delayed (≥3 beats) right to left shunt on CE with an elevated A-a gradient. SNH was defined by an elevated A-a gradient without shunt on CE. Controls were defined as patients with normal A-a gradient with or without delayed shunt on CE. We compared clinico-demographic and outcome data. Overall mortality was determined using OLT as a time varying covariate and waitlist mortality with transplant as competing event.
Results: 212 patients were included with 69 (33%) HPS, 36 (17%) SNH and 107 (50%) controls. HPS, SNH and controls were similar in all clinico-demographic features except for a higher prevalence of current or past smoking, right ventricular hypertrophy, elevated A-a Gradient and reduced DLco in the SNH and HPS groups (table). Chest imaging findings were similar in all three groups. HPS and SNH had lower FVC values than controls. Over a median follow-up of 15 months, waitlist mortality occurred in 9 (25%) patients with SNH, 18 (26%) with HPS and 9 (12%) controls. On multivariate analysis adjusting for MELD, age, gender and race, risk of waitlist mortality relative to controls was found to be 6 fold (SHR: 5.7, 95% CI: 1.61–20) higher for SNH and 5 fold for HPS (SHR: 4.9, 95% CI: 1.4–17.3) patients. Overall mortality on multivariate analysis was 3 fold higher in HPS (HR: 3, 95% CI: 1.5–6) and SNH (HR: 3, 95% CI: 1.3–6) than controls.
Conclusions: Patients with SNH have increased mortality relative to controls and similar clinical-demographic features relative to HPS. Further studies are required to ascertain if SNH represents undetected intrapulmonary shunting, extrapulmonary shunting or a distinct process that alters gas exchange.

	Controls (n = 107)	HPS (n = 69)	SNH (n = 36)	p
Age	53±9	52±9	52±10	0.8
MELD	14±5	13±4	12±5	0.06
Current/Past Smokers	64 (60%)	32 (46%)	27 (75%)	0.016
				<0.00
A–a gradient	6±5	31±14	25±14	1
DLCO predicted (corrected for HgB)	64±14	55±16	62±18	0.001
FEV/FVC	79±6	77±5	77±6	0.25
FVC% predicted	92±15	86±14	86±13	0.016
Right ventricular hypertrophy	14 (13)	19 (29)	10 (28)	0.025

P0124
AUTOLOGOUS CD34+ STEM CELL INFUSION AS A BRIDGE TO LIVER TRANSPLANT IN DECOMPENSATED CIRRHOSIS

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Background and Aims: The increasing prevalence of liver cirrhosis coupled with the paucity of donor tissues and high cost for liver transplantation, has paved the way for evaluation of cell based regenerative therapies, particularly adult hemopoetic stem cell based treatment as an alternative of bridge to liver transplant. The aim of this study was to evaluate the effect of autologus CD 34+ stem cell infusion in decompensated cirrhosis.
Methods: Patients with liver cirrhosis having MELD score >14 were included in the study and divided into 2 groups – control (n = 23) and study group (n = 22) based on opting for standard of care treatment and autologus CD34+ cell infusion respectively. The study population were not willing for immediate living donor liver transplant (LDLT) and did not wish to be enrolled in the deceased donor liver transplant (DDLT) waiting list. The patients in the control group did not have eligible donor for LDLT and were included DDLT waiting list. In the study group, Granulocyte-Colony stimulating factor (G-CSF) was given @ 5 μgm/kg body weight for 3 consecutive days followed by leukapheresis and isolation of CD34+ cells using immune magnetic separation. CD34+ cells (count 10^6-10^8) were infused through the hepatic artery under radiological guidance. Patients were monitored and discharged within 24 hours and evaluated 4 weeks and at 3 months.
Results: The procedure was safe with no treatment related side effects. There was no incidence of hepatic artery thrombosis post procedure. At 4 weeks, in the study group, the mean serum albumin showed statistically significant (2.83±0.36 vs 2.43±0.42, p = 0.001) which was however not maintained at end of 3 months. At 3 months, there was significant improvement in serum creatinine (1.01±0.32 vs 1.24±0.50, p = 0.06) and MELD score in the study group (15.73±3.35 vs 19.42±6.52 p = 0.02). There was no significant difference in the transplant free survival between the two groups, but due to the small sample size a Kaplan Meyer graph could not be drawn.
Conclusions: Autologous stem cell therapy is a safe and effective therapy which improves MELD score at end of 3 months. It can be used to delay but not as an alternative to liver transplant. It serves as a bridge to transplant and provides a window period during which transplant may be delayed. Whether repeated infusions of stem cells can further delay or improve prognosis has to be evaluated

P0125
HEPATOPULMONARY SYNDROME (HPS) SCREENING IN PATIENTS WITH CHILD A/B LIVER CIRRHOSIS

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Background and Aims: Most data about HPS prevalence come from studies in liver transplantation (LT) candidates. The aim of this study was to analyze HPS prevalence in patients with Child A/B cirrhosis, its natural history and the incidence of new cases along the follow up.
Methods: Arterial blood gases, spirometry and difussion study were done in all Child A/B cirrhotic patients without contraindications for LT who consulted in our Unit from 2007 to 2014. HPS was diagnosed in those with $P(A\text{-}a)O_2$ ≥15 and pulmonary vasodilatation showed in subsequent contrast ECO-cardiography. In those without HPS the study was repeated yearly through the follow up.

Variables recorded were: age, sex, tobacco, BMI, diabetes, HIV infection, etiology of the cirrhosis, Child and MELD scores, previous decompensations, presence of varices and platelet count. Data were analyzed by SPSS.

Results: 360 patients were studied, 286 male, mean age 52.09±7.09 years. Child score was A in 292 and B in 68 and mean MELD score 10±3.3. 152 patients were active smokers, HIV infection was present in 43, diabetes in 86 and gastroesophageal varices in 260. 213 patients had had liver decompensations previous to the inclusion. The etiology of the cirrhosis was alcohol in 238, HCV in 50, both in 33, HBV in 20 and others in 19.

171/360 (45.2%) patients had $P(A\text{-}a)O_2$ ≥15 mmHg in the first study. Contrast ECO-cardiography was done in 137 and showed pulmonary vasodilatation in 29; 27 of them had PO_2 >60 mmHg and only 2 PO_2 <60 mmHg. 21 patients with mild or moderate HPS were followed more than 6 months (mean follow-up 39.3±18.3 months;12–75) and none of them progressed to severe HPS. The incidence of new cases of HPS was 0.7 cases/100 persons-year (5/198 patients followed more than 1 year with a mean follow-up of 43.87±24.9 months; 6–113).

Patients with HPS were younger (49.9±6.2 vs 52.2±7, p = 0.08), had more frequently Child B score (37.9% vs 18.8%, p = 0.015) and had a higher MELD score (11.28±3.3 vs 10.03±3.3, p = 0.07) than those without HPS. There were no significant differences in the rest of the variables analyzed.

Conclusions: HPS prevalence in Child A/B cirrhotic patients was about 10%; most of them had mild or moderate hypoxemia, which remained stable through follow up. The incidence of new cases was low. Patients with HPS were younger and had more severe liver disease than those without it.

P0126

THE STROOP APP CAN PREDICT THE DEVELOPMENT OF OVERT HEPATIC ENCEPHALOPATHY IN A MULTI-CENTER STUDY

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Background and Aims: Testing for covert HE (CHE) is not routinely performed in part due to the unclear effect on outcomes. The Stroop App is a good test for CHE. Aim: evaluate the ability of Stroop App to predict overt HE (OHE) in a multi-center study.

Methods: Three sites [Virginia (VA), Ohio (OH) Arkansas (AR)] enrolled outpt cirrhotics (+/− OHE) and age-matched healthy controls. All subjects were given the Stroop App, psychometric hepatic encephalopathy score (PHES) and inhibitory control test (ICT). Impaired performance on PHES and ICT beyond controls was calculated to diagnose CHE as two separate gold standards to which the App OffTime+OnTime ROC analysis was compared. The cut-off with the highest AUC was used to define CHE on the Stroop App. Patients were followed for at least 6 mths till OHE development (defined as ≥ grade 2 and hospitalization). Time-dependent Cox-regression models for OHE development were created using age, baseline OHE, MELD and CHE on Stroop using either ICT or PHES as gold standards for all pts. A similar model was also tested for those without prior OHE for time to first OHE.

Results: We enrolled 387 cirrhotic patients (230 VA, 107 OH, 50 AR) and 302 controls who were used to judge impaired PHES or ICT. Patients were similar in age (57 VA, 58 OH, 56 AR, p = 0.4) but VA pts had higher %OHE (41% VA, 30% OH, 30% AR, p = 0.05). Using ICT as the gold standard (OffTime + OnTime = 181.9 seconds), 54% total (53% VA, 55% OH, 58% AR) had CHE while using PHES (OffTime + OnTime = 195.9 seconds), 39% total (40% VA, 41% OH, 26% AR) had CHE.

Entire group outcomes: On a median follow-up of 11 (IQR 8–15) mths, 13% pts developed an OHE episode (12% VA, 14% OH, 20% AR). On regression, time to OHE was predicted by MELD (HR 1.05, p = 0.03), baseline OHE (HR 1.95, p = 0.04) and Stroop impairment based on ICT (HR 2.1, p = 0.05). Stroop impairment with PHES and age were not predictive.

Without prior OHE: in the 252 pts without prior OHE, on a median follow-up of 10 (IQR 8–15) months, 9% pts developed their first OHE episode (7% VA, 9% OH, 13% AR). On regression, in the first model, time to the first OHE was predicted by MELD (HR 1.14, p = 0.0004) and Stroop impairment by PHES (HR 7.4, p = 0.0006). In the second model, Stroop impairment by ICT was also predictive for first OHE (HR 4.1, p = 0.02) independent of MELD score (HR 1.14, p = 0.0004).

Conclusions: Impaired performance on the Stroop App can predict the development of subsequent OHE episodes in patients with and without OHE at baseline independent of the MELD score in this multi-center study.

P0127

PATIENT-REPORTED HEALTH-RELATED QUALITY OF LIFE OUTCOMES INDEPENDENTLY PREDICT DEATH, TRANSPLANT, HOSPITALIZATION AND HEPATIC ENCEPHALOPATHY IN CIRRHOSIS

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Background and Aims: Patient-reported outcomes such as the measures of quality of life (QOL) in PROMIS (Patient Reported Outcomes Measurement Information System) tools are important to evaluate effectiveness of treatments. However their role in predicting outcomes in cirrhosis beyond current models is unclear. *Aim:* Analyze the additive impact of PROMIS QOLtools in predicting time to death/transplant, all hospitalizations and hepatic encephalopathy (HE)-related hospitalizations in cirrhosis

Methods: Outpt cirrhotics were administered PROMIS computerized tools for 11 domains (anger, anxiety, depression, physical function, pain behavior & impact, sleep & wake disturbances and social activities & roles). PROMIS scores, MELD score, HE status, age, sex and alcoholic etiology were then entered into a Cox-regression model for times to composite death/transplant, all hospitalizations and HE-related hospitalization. To analyze the impact beyond HE, a separate PROMIS+HE only model was also studied.

Results: 207 cirrhotics (57 yrs, MELD 12, 64% men, 29% HE, 44% HCV, 18% alcohol) were enrolled. HE pts had a higher MELD (16 vs 10, p < 0.0001) and were impaired on all PROMIS tools (p < 0.001) compared to no-HE pts except anger, anxiety and depression. *Follow-up:* Pts were followed for 38mths (IQR 22–47 mths), during which 35% had death/transplant (20% transplant/15% died), 33% were hospitalized and 12% were hospitalized for HE.

Model with all variables: *Time to death/transplant*: HE, MELD and 4 PROMIS domains (Anger, fatigue, pain behavior and wake disturbances) were significantly predictive. *Time to hospitalization*: MELD with 2 PROMIS domains (Anger, Physical function) were predictors. *Time to HE-related hospitalization*: MELD, Anger, depression, sleep & wake disturbances were predictive. Age, gender and alcohol etiology were not predictors for any outcome.

HE+PROMIS model: *Time to death/transplant*: HE with anger, fatigue, physical function, pain behavior and wake disturbance were predictors. *Time to hospitalization*: HE, anger, depression, pain impairment and physical function. *Time to HE-hospitalization*: HE,

anger, depression, sleep & wake disturbances were predictive. Thus PROMIS results add to prognostication provided by HE alone.
Conclusions: Patient reported QOL outcomes add to current prognostic models of death/transplant and hospitalizations in cirrhosis independent of HE status and liver disease severity. Inclusion of patient-reported outcomes could improve our study of cirrhosis research effectiveness and encourage inquiry into QOL issues.

P0128
LOW TESTOSTERONE LEVELS ARE ASSOCIATED WITH SARCOPENIA IN CIRRHOTIC PATIENTS

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Background and Aims: Sarcopenia is associated with increased mortality in cirrhosis. Hypogonadism has been associated with sarcopenia in several other chronic disease populations. We aimed to investigate if sarcopenia is associated with low testosterone levels in patients with cirrhosis.
Methods: A total of 211 cirrhotic patients were studied prospectively. Muscle mass was analyzed using computed tomography (CT) scans of paraspinal skeletal muscle at the level of the 3rd lumbar vertebral body (L3 SMI). Sarcopenia was defined according to a CT-based study in patients with solid tumors using optimal stratification, a statistical method similar to receiver operating characteristic (ROC) curve analysis that links specific threshold values for L3 SMI in relation to an outcome (death) [L3 SMI: ≤41 cm^2/m^2 for women and ≤53 cm^2/m^2 for men with body mass index (BMI) ≥25 $kg.m^2$ and ≤43 cm^2/m^2 in all patients with BMI <25 kg/m^2]. Morning testosterone levels were obtained in all patients: normal (nmol/L) = males: 10.3–29.5; females: 0.6–2.0. Results were described using the mean and standard deviation.
Results: 129 of 211 patients were males (55%). Cirrhosis was caused by HCV in 74 (35%), alcohol in 55 (26%), NASH/cryptogenic in 53 (25%), autoimmune liver disease in 18 (9%), HBV in 8 (4%), and other etiology in 3 patients (1%). 87 patients (41%) were sarcopenic. The mean testosterone level in males was 18.6±1.2 and 1.5±0.1 in females. 30% of males and 16% of females had levels below normal. Male sarcopenic patients had lower testosterone levels than non-sarcopenic patients (15±1 versus 22±1.2, P = 0.005); however, no significant differences in the testosterone levels were found between female sarcopenic and non-sarcopenic patients (1.29±0.2 versus 1.65±0.1, P = 0.2). There was a good correlation between the L3 SMI and the testosterone levels (r = 0.37, P < 0.001). Male gender (OR 5.26, 95% CI 2.13–12.98, P < 0.001) and testosterone levels (OR 0.95, 95% CI 0.91–0.99, P = 0.02) were associated with sarcopenia by multivariate regression analysis; but age, MELD score, and etiology of the cirrhosis were not.
Conclusions: Lower testosterone levels are associated with sarcopenia in male cirrhotic patients. Testosterone replacement therapy is a potential therapeutic strategy to improve muscle mass in male cirrhotic patients. The safety and efficacy of this treatment requires prospective evaluation.

P0129
PORTAL HYPERTENSION AND COLLATERAL CIRCULATION CAN INFLUENCE ON THE ESTIMATION OF LIVER STIFFNESS MEASUREMENT BY TRANSIENT ELASTOGRAPHY

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Background and Aims: Recently, some studies showed the relationship between LSM and hepatic venous pressure gradient (HVPG). However the influence of portal hypertension and secondary liver congestion by right heart preload increase has not been known. So, in this study, we evaluated the relationship between the change of LS and HVPG (ΔLS and ΔHVPG) after 3 months propranolol treatment and the change of strength of relationship between LS and histologic grade.
Methods: LSM and HVPG were performed at baseline and after 3month propranolol treatment in 61 consecutive cirrhotic patients [male, 52 (85.2%)] who had biopsy proven cirrhosis with HVPG≥12 mmHg were included. Linear regression analysis was performed for evaluation of relationship between ΔLS [%, (baseline LS − follow-up LSE)/baseline LS × 100] and ΔHVPG [%, (baseline HVPG − follow-up HVPG)/baseline HVPG × 100].
Results: The baseline mean HVPG and LS were 17.3±4.1 mmHg (12–27) and 45.3±18.3 kPa and these showed significant correlation ($r^2 = 0.24$, $p < 0.0001$). Follow up HVPG and LS [13.0±4.8 mmHg (4–21) and 33.3±17.4 kPa] after propranolol treatment also showed significant correlation ($r^2 = 0.46$, $p < 0.0001$). A strong positive relationship between ΔLS (%) and ΔHVPG (%) was also observed in the overall population ($r^2 = 0.34$, $p < 0.0001$). Thirty-three patients (37/61, 60.5%) were propranolol responders. In responder group, baseline LS correlated with the baseline HVPG ($r^2 = 0.29$, $p < 0.001$) and it more closely correlated with the HVPG after propranolol treatment ($r^2 = 0.58$, $p < 0.001$) but there was no correlation in nonresponders. Baseline LS correlated with the Laennec histologic grades ($r^2 = 0.27$, $p < 0.001$) and it showed more close correlation with histologic grades ($r^2 = 0.37$, $p < 0.001$) after propranolol treatment. In responder group, LS showed more significant improved correlation with the histologic grades ($r^2 = 0.27$ vs. $r^2 = 0.40$, $p < 0.001$) after propranolol treatment, however there was no significant change in nonresponder ($r^2 = 0.23$ vs. $r^2 = 0.27$, $p > 0.05$).
Conclusions: The interval change of LS showed significant correlation with the change of HVPG after propranolol treatment. Improved correlation of adjusted LSM by propranolol treatment with histologic grades suggested that LS is also influenced by portal hypertension in patients with clinically significant portal hypertension.

P0130
VITRO-SCORE (vWF-Ag/THROMBOCYTES) PREDICTS MORTALITY IN PATIENTS WITH LIVER CIRRHOSIS INDEPENDENT OF CHILD–PUGH STAGE AND MELD SCORE

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Background and Aims: vWF-Ag and VITRO Score are promising non-invasive markers in diagnosis and prognosis of liver cirrhosis. vWF-Ag levels are elevated in patients with liver cirrhosis and vWF-Ag shows adequate performance in predicting CSPH and mortality in cirrhotic patients. VITRO-score shows good

performance in predicting cirrhosis in chronic HCV patients. We hypothesized that VITRO-score can predict mortality in a cohort of cirrhotic patients.

Methods: We collected and analysed data of 294 cirrhotic patients. Routine clinical parameters and vWF-Ag levels were measured and VITRO-Score (vWF-Ag/platelets) was calculated. During follow-up, cases of transplantation or death were recorded. Descriptive analysis was performed to describe the baseline characteristics of the cohort. Furthermore we performed Kaplan–Meier analysis to detect differences in survival between vWF-Ag, VITRO and MELD.

Results: male 194 (66%), median age 60 (52–68; IQR); aetiology of liver disease: Hep C 44.22%, ALD 29.59%, NASH 9.86%, other or unknown 16.32%. 64.60% were categorized as CPS A, 29.30% as CPS B and 6.10% of our cohort as CPS C. According to D'Amico 187 (63.60%) were compensated.

Median follow-up time was 21 months (7–35; IQR).

73 (24.80%) patients died during follow-up or were censored due to liver transplantation. Overall median transplant-free survival time was 33 months with a 1-year survival rate of 84%. 25% mortality was reached after 26 months. Patients with VITRO-score ≥2.5 show significantly worse survival with a one-year mortality of 22% and 25% mortality after 14 months compared to a one-year mortality of 10% and a 25% mortality after >42 months in patients with VITRO-score <2.5 (log-rank <0.001).

Median VITRO-score is significantly higher in patients who died with 4.34 (1.91–5.40; IQR) compared to survivors with 2.47 (1.24–3.24; IQR) ($p < 0.001$).

VITRO equals MELD in mortality prediction with an AUC of 0.68 (0.62–0.74 95% CI) for VITRO and an AUC of 0.65 (0.59–0.71 95% CI) for MELD, ($P > 0.05$).

Conclusions: A VITRO-score cut-off of 2.5 is able to detect different survival in a cohort of cirrhotic patients independent of CPS and Meld-score.

VITRO Score as a predictor of mortality could be used as an easy accessible prognostic tool in clinical practice and may help to identify patients at risk and lead them to adequate treatment.

P0131

BACTERIAL DNA QUANTIFICATION IN ASCITES: A NOVEL TOOL TO DEFINE HIGH RISK PATIENTS?

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Background and Aims: Spontaneous bacterial peritonitis (SBP) represents a serious complication in patients with liver cirrhosis. Culture-based methods to detect bacteria in the ascites fluid showed limited sensitivity, and the diagnosis of SBP is therefore usually made by the surrogate of an increased total ascites leukocyte or neutrophil count. Culture-independent 16S rRNA-gene based amplification methods allowed for the detection of bacterial DNA (bactDNA) in both neutrocytic and non-neutrocytic AF but the significance of this novel tool is still debated. In the current study the impact of bactDNA quantification in ascites on the clinical outcome of patients with cirrhosis was evaluated.

Methods: AF-samples of 173 cirrhotic patients were collected between February 2011 and December 2012. BactDNA was quantified by using real-time PCR with broad range primers targeting the V3 and V4 variable region of 16S rRNA-Gene. Positive AF-samples were sequenced and chromatograms were identified using RipSeq. The detection of bactDNA in AF was correlated with routinely recorded clinical parameters and survival.

Results: BactDNA was detected in 57/144 (39.6%) non-leukocytic AF and 10/23 (43.5%) of leukocytic AF ($p = 0.724$). The median level of bactDNA was significantly lower in non-leukocytic than in leukocytic AF (5.7×10^2 copies/mL vs. 1.2×10^4 copies/mL, $p = 0.008$). The detection of a bactDNA level above the quantification limit of the assay but not the presence of leukocytic AF was significantly associated with a reduced 180-day survival [180-day survival rate 42.6% vs. 60.9% ($p = 0.030$)]. The bacterial spectrum detected by molecular methods was dominated by gram-positive strains such as Staphylococcus, Streptococcus, and Enterococcus species.

Conclusions: The presence of quantifiable concentrations of bacterial DNA in patients with non-leucocytic ascites fluid samples using culture-independent 16S rRNA-gene based methods may help to define a new risk group with reduced survival. Sequence analyses confirmed a shift to gram positive and multibacterial strains. Pre-emptive antibiotic therapy in bactDNA positive patients should be investigated in further prospective interventional trials.

P0132

EXTERNAL VALIDATION OF THE CLIF-SOFA IN CIRRHOTIC PATIENTS ADMITTED TO INTENSIVE CARE UNITS (ICUS): A META-ANALYSIS

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Background and Aims: The prognostic performance of the Chronic Liver Failure-Sequential Organ Failure Assessment (CLIF-SOFA) score in cirrhotic patients admitted to Intensive Care Units deserves large external validations. The aim of this meta-analysis was to assess the ability of the CLIF-SOFA to predict in-ICU, in-hospital, and 6-month mortality in ICU survivors.

Methods: The CLIF-SOFA was computed retrospectively in 6 studies including 1221 cirrhotic patients admitted between 1995 and 2012. Studies have been selected by the participation of the corresponding authors who responded to a standardized questionnaire. The prognostic performance of different cutoffs of the CLIF-SOFA to predict mortality at different timepoints was assessed by the weight-adjusted odds ratios and positive predictive values and compared to that of the SOFA, the modified SOFA (mSOFA) and the MELD.

Results: On admission, 72.1% of patients ($n = 880$) had a CLIF-SOFA ≤14 and only 1.1% ($n = 13$) had a CLIF-SOFA >22. Among all the available prognostic scores, the best predictor of in-ICU mortality was a CLIF-SOFA ≥22 (OR = 5.94, 95% CI 1.71–20.64; $p = 0.005$; PPV = 1.00), followed by a SOFA >19 (OR = 10.37, 95% CI 5.65–19.01; $p < 0.001$; PPV = 0.94). Predictive value of in-ICU mortality was better for a CLIF-SOFA ≥15 (OR = 7.44, 95% CI 3.43–16.12; $p < 0.01$; PPV = 0.81) than for an increased SOFA on day 3 (OR = 4.75, 95% CI 2.33–8.97; $p < 0.001$; PPV = 0.71) or a MELD score ≥26 (OR = 5.37, 95% CI 4.01–7.21; $p < 0.001$; PPV = 0.66). Prognostic value of in-hospital mortality was good for a CLIF-SOFA ≥15 (OR = 3.93, 95% CI 2.13–7.26; $p < 0.001$; PPV = 0.88), for a mSOFA >13 (OR = 11.92, 95% CI 4.59–30.94; $p < 0.001$; PPV = 0.94) and a SOFA >19 (OR = 11.56, 95% CI 3.23–41.32; $p < 0.001$; PPV = 0.94). Among ICU survivors, 6-month mortality was still predicted by a CLIF-SOFA ≥22 (OR = 7.43, 95% CI 1.18–46.62; $p = 0.032$; PPV = 1.00), and a MELD ≥26 (OR = 3.97, 95% CI

1.92–8.22; p < 0.001; PPV = 0.75), whereas high values of SOFA and mSOFA did not provide any significant prediction.
Conclusions: In critically ill cirrhotic patients, the CLIF-SOFA is able to predict both in-ICU mortality and 6-month mortality in ICU survivors, conversely to the SOFA and mSOFA. High values of CLIF-SOFA better predict in-ICU mortality than high values of SOFA or increase in SOFA on day three, and better predict 6-month mortality in ICU survivors than the MELD score. The CLIF-SOFA thus appears as the prognostic score of choice for the critically ill cirrhotic patients.

P0133
NUTRITION AND PHYSICAL THERAPY TARGETS ARE NOT BEING MET IN ADMITTED PATIENTS WITH CIRRHOSIS
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Background and Aims: Low muscle mass and frailty are major determinants of morbidity and mortality in cirrhosis. Adequate nutritional therapy and early mobilization can impact these parameters. Current cirrhosis guidelines target daily caloric intake at 35–40 kcal/kg and protein intake at 1.2–1.5 g/kg. There are no formal guidelines for mobilization therapy in cirrhosis.
Aims: In hospitalized cirrhotic patients, to evaluate the adherence to nutritional guidelines and determine the proportion of cirrhotic patients who had early (within 72 h of study enrollment) mobilization therapy.
Methods: Multicenter prospective trial involving 3 hospitals (2 Alberta, 1 Ontario) with data collection over a 13-month period (June 2013-July 2014). All patients had cirrhosis and were recruited within 72 hours of admission. Patients with non-hepatocellular carcinoma related malignancy, palliative HCC, severe end-organ failure (eg. Dialysis, COPD on home oxygen, CHF) or Hepatic encephalopathy Conn's score >1 were excluded. Patients were followed for 30 days from initial assessment or until discharge from hospital. Detailed nutritional and mobilization data was collected over the first 3 days of assessment and at two centers, also at ~day 8.
Results: 137 patients were recruited. Baseline characteristics – Mean age 55.5 (SD: 10.3), 59% male, 77% Caucasian, mean MELD and CP scores 18.2 (6.6) and 9.5 (2.0) respectively. The top three etiologies of liver disease were alcohol (41%), HCV (33%) and autoimmune/cholestatic liver disease (12%). Eighty-two percent had at least mild ascites. The mean estimated dry weight BMI was 24.6 (5.3). By day 3, only 7% and 20% respectively met guideline based calorie and protein targets. In the 44 patients with ~day 8 data still only 11% and 14% of patients met calorie and protein targets respectively. A subset of 77 patients had mobilization data available. Over days 1–3, 22.1%, 38% and 55% of these patients were able to mobilize/be mobilized from their bed to outside of their hospital room. Only three patients had a formal physiotherapy consult.
Conclusions: There is an opportunity to improve on the early nutritional and mobilization therapy in cirrhotic inpatients. Less than 20% of patients met nutritional intake recommendations by day 3 of study enrollment and only 4% received a formal physiotherapy consult. National inpatient cirrhosis care guidelines and standardized orders would allow us to determine whether optimization of these variables can impact clinical outcomes in cirrhosis.

P0134
COMPLICATIONS OF ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY IN PATIENTS WITH CIRRHOSIS
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Background and Aims: ERCP, which is commonly performed in cirrhotic patients with common bile duct stones and strictures, may be associated with significant complications such as pancreatitis, cholangitis, and bleeding. Patients with cirrhosis are at risk of bleeding and infectious complications after ERCP, but the incidence and risk factors in this population is not well described. The aim of this study was to examine the incidence and specific risk factors of post-ERCP complications in patients with cirrhosis.
Methods: We evaluated all ERCP's performed in patients with cirrhosis at Hospital Clinic, Barcelona from 2002–2013. We also analyzed a matched control group of non-cirrhotic patients with ERCP in the same period. Patient and procedure-related risk factors were analyzed. Predictive factors for post-ERCP complications were determined by univariate and multivariate analysis
Results: 256 ERCP's were analyzed (85 in cirrhotic and 171 in non-cirrhotic patients). Etiology of cirrhosis: HCV and alcohol (98%), median MELD 12, Child A/B/C (33%, 50%, 17%). Complications occurred in 12 patients (14%) in the cirrhosis group (11 mild, 1 severe): pancreatitis 3 cases (3.5%), cholangitis 3 cases (3.5%), and post-sphincterotomy bleeding 6 cases (7%). The overall rate of complications was significantly higher in patients with cirrhosis compared to non-cirrhotic patients (14% vs 6.4%, P = 0.04). Only bleeding occurred more commonly in patients with cirrhosis compared to non-cirrhotics [6/85 (7.0%) vs 3/117 (1.7%), P = 0.03]. The incidence of pancreatitis and cholangitis were not significantly different among both groups. Logistic regression identified sphincterotomy [OR 8.99 (CI 1.08–74.60), P = 0.04] and cirrhosis [OR 3.51 (CI 1.11–11.09), P = 0.03] as independent risk factors of post-ERCP complications, whereas cirrhosis was the only independent risk factor of post sphincterotomy bleeding [OR 4.12 (CI 1–16.93), P = 0.04].
Conclusions: The rate of complications after ERCP in patients with cirrhosis (14%) is higher than that of patients without cirrhosis. The majority of complications are mild and due to post-sphincterotomy bleeding. Cirrhosis and sphincterotomy are risk factors of post-ERCP complications. Further prospective studies that implement preventive strategies to avoid bleeding in patients with cirrhosis are needed

P0135
IMPACT OF VASOPRESSIN AVP1A RECEPTOR GENE POLYMORPHISMS ON MORTALITY AND RENAL FAILURE IN PATIENTS WITH ACUTE DECOMPENSATION OF CHRONIC LIVER DISEASE
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Background and Aims: Acute-on chronic liver failure (ACLF) is defined as an acute decompensation (AD) of former stable liver disease accompanied by organ failure and a high short-term

mortality. Systemic hemodynamic dysfunction and activation of endogenous vasoconstrictor systems are thought to contribute to the pathogenesis. Vasopressin is a key-regulator in hemodynamic homeostasis. Aim of the present study was to assess in patients with AD whether genetic variation of the human arginine vasopressin 1A receptor (AVP1aR) is associated with increased mortality or the development of ACLF, renal or circulatory failure.

Methods: Eight single nucleotide polymorphisms (SNPs) of AVP1aR with possible clinical relevance were identified. From 188 cirrhotic patients hospitalized for AD, clinical, laboratory and survival data from the CANONIC database were used. Presence of ACLF was defined according to the modified CLIF-sequential organ failure assessment (SOFA) score. All patients were genotyped for all eight SNPs using polymerase chain reaction followed by restriction fragment length polymorphism or PCR allele-specific amplification primers. Fisher's exact test and linear regression analysis were used to test for associations between genetic frequencies and dichotomous and continuous variables respectively. Cox proportional hazard analysis adjusted for age was performed to assess the association between AVP1a SNPs and mortality at set time points. $P < 0.05$ was considered significant.

Results: Ninety-three patients without ACLF and 95 patents with ACLF were included. As expected, mortality rate at 12 months of follow-up (FU) was higher in patients with ACLF (59%) than in patients with AD who did not develop ACLF (28%). Mutations in rs7298346 [HR 1.81 (95% CI 1.02–3.23), $p = 0.044$] and rs7308855 [HR 2.17 (1.17–4.01), $p = 0.013$] showed a clear trend towards a lower overall survival in all patients at 90 days of FU. For rs 7308855 this trend persisted until 180 days of FU [HR 1.87 (1.00–3.50), $p = 0.051$]. In addition, T>A mutation in rs7298346 was associated with the presence of renal failure in patients with ACLF ($p = 0.025$). No association was found between AVP1a SNPs and the occurrence of ACLF or circulatory failure.

Conclusions: There is a clear trend of AVP1a SNPs to be associated with increased mortality in patients with acute decompensation of liver disease and with the occurrence of renal failure in ACLF.

P0136

ALFAPUMP FOR THE TREATMENT OF REFRACTORY ASCITES IN CIRRHOTIC PATIENTS

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Background and Aims: The automated low flow ascites pump (**alfa**pump) is a novel treatment for the management of refractory ascites in patients contraindicated for or with an ineffective TIPS. The aim of this study was to monitor safety and clinical performance.

Methods: From September 2012 data from patients with an alfapump have been collected in an international multicentre prospective registry. Inclusion criteria were refractory or recurrent ascites with no other treatment option than repetitive paracentesis, age >18 years, informed consent, ability to manage the alfapump system. Data points were analysed at baseline before surgery, perioperatively, and after 1, 3, 6, 12 and 18 months during follow-up.

Results: In total, 55 patients were included (76% men, 24% women), mean age was 61 (range 44–78). Aetiology of cirrhosis was alcohol (69.1%), chronic hepatitis C virus infection (9.1%), NASH (5.5%) and cryptogenic (5.5%).

The median duration of the implantation was 60 min (25th/75th percentile: 50/70) and the median length of hospital stay after implantation was 6 days (3/12). The number of large volume paracenteses per month decreased from 2.17 (1.4/4.3) to 0.0 (0.0/0.43), the volume of each paracentesis decreased from 7.0 litres (5.0/9.0) to 4.6 litres (3.0/6.0). The median volume of ascites pumped was 0.9 litres (0.7/1.1) per day.

The most frequent complication was obstruction of the peritoneal catheter in 11 patients, followed by pump failures due to technical problems in 7 patients and infections in 6 patients. Of 55 patients, 17 are ongoing, 18 died, 8 were transplanted, 10 were withdrawn, 1 completed the study and 1 was lost to follow-up. Mean survival was 6.33±5.33 months with a 6 month actuarial survival of 74%. Mean BMI was 25.3 kg/m^2 at baseline and 26.1 kg/m^2 at 18 months. Serum creatinine levels increased during follow-up from 98 μmol/L (86/117) to 124 μmol/L (107/206) at 18 months. Albumin was administered in 34.5% of patients post implant in the context of paracentesis. Median serum albumin decreased from 30.0 g/L at baseline to 25.0 g/L at 18 months. Mean Child and MELD scores were 8.8 (± 1.4) and 13.4 (± 4.3) at baseline and 8.0 (± 1.4) and 12.7 (± 4.4) at 18 months, respectively.

Conclusions: Implantation of alfapump was a safe procedure in patients with refractory ascites due to advanced cirrhosis and was associated with a marked reduction in the need for paracentesis and albumin.

P0137

SAFETY OF BOCEPREVIR-BASED TRIPLE THERAPY IN HCV CIRRHOTIC PATIENTS AWAITING LIVER TRANSPLANTATION. ANALYSIS FROM A FRENCH MULTICENTER, OPEN-LABEL STUDY (ANRS HC29 BOCEPRETRANSPLANT)

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Background and Aims: To analyze the safety of a boceprevir (BOC)-based triple therapy in HCV cirrhotic patients (pts) on waiting-list (WL) for liver transplantation (LT) in a French multicenter, open-label study.

Methods: HCV genotype 1 pts received PEG-IFN-α2a 180 μg/week (W) or PEG-IFN-α2b 1.5 μg/kg/W, weight-based RBV 1000–1200 mg/d during 48W, and BOC 800 mg/8 hours from W4 to W48 or until LT. Biological and clinical safety was assessed during treatment and 6 months after the end of treatment (EOT). Results on severe adverse events (SAE) occurrence are reported.

Results: 51 pts from 15 centers were included (80% males, median age 56 years). Hepatocellular carcinoma (HCC) was present in 59%

of pts, end-stage liver disease in 41%. At inclusion, the median MELD score was 9 (range 6–18); the Child–Pugh score was A in 65% and B in 35%. Treatment median duration was 13W. Therapy was discontinued because of SAE in 20 (39%) or virological inefficacy in 12 (24%). Seven pts (14%) died after a median duration of treatment of 9W because of sepsis (n = 4), HCC progression (n = 1), haemorrhagic shock (n = 1) and cerebral haemorrhage (n = 1). Three deaths were considered as possibly related to treatment. 114 SAE were observed in 43 pts (84%). Most common diagnoses were neutropenia (25%), GGT (20%) or AST elevation (12%), anemia (10%), thrombocytopenia (10%), hepatic encephalopathy (10%) and leucopenia (10%). Severe anemia (Hb <8 g/dL) was observed in 4%. Growth factors were used in 36 pts (71%), erythropoietin, thrombopoietin and G-CSF in 23, 9 and 11 pts, respectively. Undetectable HCV-RNA 24 weeks after the EOT (SVR24) was observed in 8 pts (16%). A LT was performed in 18 pts (35%) after a mean duration on WL and of treatment of 59 and 13 W, respectively. HCV RNA was undetectable in 19% at LT and was still negative after LT in 2 pts (W24 post-LT). Severe infection occurred in 12 pts (24%) including septicemia (n = 7), ascites infection (n = 3) and urinary infection (n = 1). One pt had multiple infections. Child–Pugh score B, low Hb, lymphocytes and albumin levels at baseline were associated with the occurrence of severe infection but multivariate analysis indicated that only low albumin level (<35 g/l) remained an independent predictive factor.

Conclusions: Safety of PEG-IFN/RBV/BOC combination is poor in pts awaiting LT, with a high risk of developing severe infection. Moreover, the limited efficacy confirms the necessity of IFN-free combinations in these pts.

P0138

THE EFFECT OF TREATMENT FOR HEPATIC ENCEPHALOPATHY WITH NONABSORBABLE DISACCHARIDES ON MORBIDITY AND MORTALITY IN PATIENTS WITH CIRRHOSIS: SYSTEMATIC REVIEW AND META-ANALYSES

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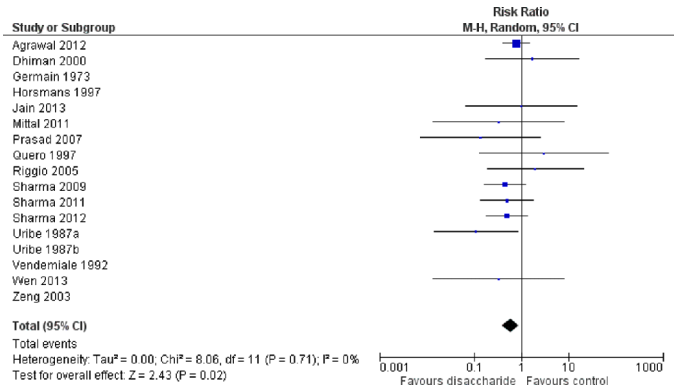
Background and Aims: The non-absorbable disaccharides (NADs), lactulose and lactitol, have been used to treat hepatic encephalopathy (HE), in patients with cirrhosis since 1966. However, a Cochrane systematic review concluded that there was insufficient high-quality evidence to support their use (Als-Nielsen *et al*, 2004). The advent of several new treatment trials requires this to be reappraised. The aim of this study was to assess the beneficial and harmful effects of NADs *vs*. placebo/no intervention in patients with cirrhosis and HE.

Methods: Randomized controlled trials published between 1966 and October 2014 were identified using a language/publication unrestricted electronic search of five data-bases; (ii) a manual search of relevant journals, symposia/conference proceedings; (ii) personal communications with investigators; and (iii) publication cross-referencing. Bias control was assessed using the Cochrane Hepato-Biliary Group domains; key outcome data were extracted. Sources of heterogeneity were sought. Small study effects, random and systematic errors were assessed and futility evaluated in sensitivity, subgroup, regression and trial sequential analyses.

Results: In total, 37 trials involving 1628 patients with overt HE (12 trials), minimal HE (14 trials) or else for primary/secondary prophylaxis (11 trials) were identified; the mean duration of treatment was 3 months. Compared with placebo/no intervention, use of NADs had a beneficial effect on HE (RR 0.56; 95% CI 0.47–0.68; participants = 1263; I^2 = 51%, 20 trials) both when used to treat the syndrome (RR 0.62; 95% CI 0.48–0.80) or else to prevent its occurrence (RR 0.47; 95% CI 0.37–0.59). The result was confirmed in analyses of high-quality trials. Use of NADs was associated with a reduction in mortality (RR 0.60; 95% CI 0.40–0.91; figure) and the risk of serious adverse events including severe infections and liver-related complications (RR 0.47; 95% CI 0.36–0.62); the risk of diarrhoea and bloating was increased. There were no differential effects in relation to the type of HE or use for treatment or prophylaxis; no clear differences were identified in treatment efficacy or safety between lactulose and lactitol. Overall the sample size was adequate and the findings statistically robust.

Conclusions: In patients with cirrhosis, NADs are beneficial for both the treatment and prevention of HE; their use is also associated with a significant reduction in liver-related morbidity and mortality.



P0139

PATIENTS EXPERIENCING REPEATED EPISODES OF HEPATIC ENCEPHALOPATHY HAVE INCREASING RISK OF DEATH. A POST HOC ANALYSIS OF RIFAXIMIN-α OPEN LABEL STUDY DATA

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Background and Aims: Hepatic encephalopathy (HE) is a chronic complication of cirrhosis. In recurrent, overt, episodic HE, which is the most common subcategory, its seriousness is due to the chronic debilitating effects of the recurrent episodes. The aim of this study was to characterise the impact of the number of prior HE episodes on the risk of death.

Methods: A post-hoc analysis was carried out using data from 322 patients with a history of HE from a phase 3, open-label study evaluating the long-term safety and tolerability of rifaximin-α 550 mg BID. All eligible patients had a Conn score of 0–2 at enrolment, and either successfully participated in a previous HE study with rifaximin-α (RFHE3001) or were new patients enrolled with ≥1 verifiable episode of HE within the preceding 12 months.

Results: 321 of 322 patients (650 observations) aged ≥18 years had all the necessary information required for analysis. The median duration of follow-up was 17 months (IQR 8.9–25.4). Stratifying patient observations by number of prior HE episodes and using the Kaplan–Meier method the year one survival was 0.947 (95% CI; 0.891–1.000), 0.898 (0.845–0.953) and 0.793 (0.735–0.856) and the year two survival was 0.910 (0.822–1.000), 0.808 (0.719–0.909) and 0.783 (0.677–0.0.828) for 'one', 'two' and 'three or more' prior HE episodes, respectively. Plotting the Kaplan–Meier curves of time to death, stratified by number of prior HE episodes, a clear association between decreased time to death and increased number of prior HE

episodes. Using log-rank tests there was no significant difference between the survival curves of one prior and two prior HE episodes [$\chi^2 = 0$ on 1 degree of freedom (d.f.), $p = 0.899$], however there were significant differences between survival curves of one prior or two prior episodes and greater numbers of prior episodes ($\chi^2 = 64$ on 2 d.f., $p < 0.001$).
Conclusions: As the number of prior HE episodes increases, the risk of death increases. In addition to impact on quality of life and health service utilisation, HE events may represent a prognostic marker of mortality.

P0140
FRAILTY ASSESSMENT IN CIRRHOSIS – VARIABLE PREVALENCE ACROSS SCREENING TOOLS
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Background and Aims: Frailty is an important predictor of morbidity and mortality in cirrhosis, and can be assessed using multiple screening tools. Some of these tools are time-consuming for use in daily clinical practice, while others ignore important domains of cognitive function and mood. Moreover, a comparison of the prevalence of frailty and its association with outcomes using different assessment tools has not been conducted. In a prospective cohort of patients with cirrhosis, we aimed to evaluate (i) the prevalence of frailty using different frailty tools, (ii) the most commonly affected frailty domains and (iii) the impact of frailty on clinical outcomes.
Methods: Data was gathered prospectively on patients with cirrhosis seen in liver clinics in Edmonton and Calgary, the majority recruited during work-up for transplant. We examined frailty using the short physical performance battery (SPPB), the Clinical Frailty scale and the 5 and 7 variable Fried frailty criteria.
Results: Of the 296 included patients, 66% were male with a mean age of 57 years (SD 9.3), MELD score of 12 (4.9) and Child Pugh score of 7.5 (2.1). The top three etiologies of liver disease were Hepatitis C (34%), Alcohol (32%) and NAFLD/cryptogenic (17%). Seventeen percent of patients had HCC. The prevalence of frailty was 7% using the SPPB, 19% using a score of ≥5 on the Clinical Frailty scale, 36% by the 5 variable Fried physical frailty criteria and 55% if cognition and depression were also evaluated as part of the 7 variable Fried criteria. The most commonly affected domains were: cognition (62%), low physical activity level (49%), exhaustion (46%), weight loss (45%), weakness (45%), depression (32%), slowness (20%). Using logistic regression analysis, all of the frailty criteria (with the exception of the SPPB) were independent of liver function in predicting admission to hospital within 6 months of assessment (subset of 79 patients). The strongest association was seen using the 7 variable Fried criteria: OR: 8.9 (2.7 to 28.9), $p = 0.001$.
Conclusions: The prevalence of frailty varies widely depending upon the choice of screening tool, ranging from 7% to 55%. In our preliminary analysis of impact on clinical outcomes, all frailty tools (apart from the SPPB) were strongly predictive of the need for hospitalization. Both physical and cognitive/mood domains of frailty were affected, and should be considered when screening patients with cirrhosis for frailty or risk of hospitalization.

P0141
BLOOD–BRAIN BARRIER DYSFUNCTION ASSESSED BY PROTEIN S-100 BETA LEVELS IN CIRRHOTIC PATIENTS
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Background and Aims: The protein S-100 beta (PS-100) is a small dimeric cytosolic protein synthetized in astrocytes and Schwann cells. High serum levels of PS-100 are associated with brain lesions and altered blood–brain barrier permeability in traumatic brain injury, ischemic stroke, cerebral tumors and subarachnoid hemorrhage. Early brain damage has been detected in patients with cirrhosis. We hypothesized that those insults could be detected by the PS-100 serum levels (normal values <0.10 μg/L). Hence, the aim of the present study was to assess PS-100 levels in cirrhotic patients, and compare them to PS-100 levels in non-cirrhotic patients followed up in the same department for initial workup of elevated liver enzymes (controls).
Methods: We prospectively included a population of cirrhotic patients admitted for complication of cirrhosis in ICU and controls. Baseline data were recorded and levels of ammonemia and PS-100 determined at admission. Overt HE was diagnosed as a West-Haven score ranging between 2 and 4.
Results: Between October 2013 and October 2014, 124 cirrhotic patients (mean age: 63 years, male gender: 73%, etiology of cirrhosis: alcoholic 51%, viral 25%, other 24%, Child–Pugh 11 [8–12], MELD score 21 [14–26]) and 79 non-cirrhotic patients (mean age: 63 years, male 59%, etiology of elevated liver enzymes: alcoholic 5%, viral 44%, NASH 38%, other 13%, mean Fibroscan: 5.3 kPa, mean FibroTest: 0.15) were included. Among cirrhotic patients, 40 patients (33%) displayed HE at admission and 50 (41%) had an infection. Mean PS-100 level was 0.15±0.01 for cirrhotic patients and 0.07±0.01 for non-cirrhotic patients ($p < 0.0001$). In cirrhotic patients, PS-100 was correlated to AST ($p = 0.0027$), bilirubin ($p = 0.0001$), Child–Pugh score ($p = 0.001$) and MELD ($p = 0.0004$) and inversely correlated to albumin ($p = 0.04$), sodium ($p = 0.01$), PTT ($p = 0.001$), and FV ($p = 0.011$). PS-100 levels were not correlated to the presence of overt HE nor to infection. In multivariate analysis, levels of PS-100 were independently associated with MELD score ($p = 0.0006$), whereas overt HE was associated to hyperammonemia ($p = 0.002$) and the presence of infection ($p = 0.008$) but not to PS-100 levels.
Conclusions: Patients with cirrhosis display neurological insult or blood–brain barrier dysfunction even in the absence of overt HE. Brain damage is more frequent in patients with advanced liver disease.

P0142
MORTALITY RISK FACTORS IN CIRRHOTIC PATIENTS IN INTENSIVE CARE UNIT: A MONOCENTRIC RETROSPECTIVE STUDY AT BESANÇON'S UNIVERSITY HOSPITAL FROM 2002 TO 2014
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Background and Aims: The prognostic in cirrhotic patients admitted in intensive care unit (ICU) is poor and those who will benefit from intensive care should be identified at early as possible. Objectives of this study were to assess the predictors of mortality in cirrhotic patients admitted in our medical ICU and to evaluate the relevance of a new prognostic score measured at baseline.
Methods: Retrospective cohort study including all cirrhotic patients consecutively admitted in our intensive care unit between January

2002 and May 2014. 170 clinical and paraclinical variables were collected for each patient. Multivariate analyses on survival used the logistic regression for in-ICU survival and Cox model for 28-day and 6-month survival.
Results: Of the 7427 admissions recorded during the study period, 258 (3.5%) concerned 218 cirrhotic patients (165 men; median age: 59). Alcoholism was the main cause of cirrhosis (85%). On admission, the medians of the different prognostic scores were as follows: Child–Pugh=11, MELD=28, SOFA=12, modified SOFA=10 and IGS II=59. 196 (90%) patients received mechanical ventilation; 187 patients (86%) received catecholamines and 112 (51%) required renal replacement therapy (RRT). The in-ICU, 28-day, 3-month and 6-month mortality rates were 47% (n = 103), 49% (n = 107), 62.5% (n = 130) and 66% (n = 136), respectively. Among the 115 patients who were discharged from ICU, only 7 patients underwent transplantation, whereas 48 had no clear contraindication. The best prediction of in-ICU (AUROC=0.94) and 28-day (AUROC=0.89) mortality was provided by the variation of SOFA between baseline and day 3. Multivariate analyses on in-ICU mortality identified 3 independent variables, incorporated in a new 3-variables prognostic model as follows: SOFA≥12 (OR = 5.8 [95% CI: 2.9–11.6]: 2 points), INR≥2.6 (OR = 1.7 [1.2–3.3]; 1 point) and RRT (OR = 4.2 [1.9–9.2]; 1 point). Those variables were also able to discriminate 28 days, 3 months and 6 months survivors. For a value of the 3-variables prognosis score at 4 (16% of patients), the prediction of in-ICU mortality was excellent (PPV = 91%), similar to that given by delta SOFA (PPV = 93%).
Conclusions: The prognostic assessment tools already available are effective, but are improved by using a new score grouping the SOFA, INR, and RRT. This score enabled us to identify patients in which the assessment on day 3 is not mandatory for predicting in-ICU mortality.

P0143
IMPULSIONAL, POINT AND BIDIMENSIONAL SHEAR WAVE ELASTOMETRY FOR PORTAL HYPERTENSION: SAME STIFFNESS THRESHOLDS?

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Background and Aims: Liver stiffness (LS) values are becoming tested in chronic liver disease to investigate the presence of portal hypertension (PH). LS measured with Transient elastography (TE) correlates with HVPG, and 21.1 kPa proposed as best cut-off to predict the presence of clinically significant PH (CSPH). New elastographic techniques which may overcome some limitations of TE became recently available, but their diagnostic accuracy remain to be defined. We aimed to compare the performance of TE, point shear wave elastography (ElastPQ) and 2D-Shear Wave Elastography (2D SWE) to predict the grade of PH in a cohort of cirrhotic patients.
Methods: 65 consecutive patients (63.1%M, 60.2y) with chronic liver disease submitted to HVPG measurement and at least one elastographic technique, (TE based on impulsional elastography and/or ElastPQ – on a Philips IU22, and/or 2D SWE – on a Aixplorer by Supersonic Imagine) blindly each from another technique, were enrolled between December 2013 and November 2014.
Results: 44/65 patients (67.7%) had a baseline HVPG >10 mmHg, thus classified as CSPH. 40 patients (61.5%) underwent TE, 31 (47.7%) ElastPQ, 25 (38.5%) 2D-SWE measuring liver stiffness.
HVPG was well correlated with either TE (r = 0.695; p < 0.0001), ElastPQ (r = 0.684; p = 0.001), or 2D-SWE (r = 0.762; p < 0.0001).
The overall concordance between elastographic techniques (tested in 51 patients who underwent either 2 or 3 methods) was only moderate (ICC = 0.409). TE was concordant with both other techniques (ICC = 0.772 for ElastPQ, ICC = 0.754 for 2D-SWE), while ElastPQ and 2D-SWE were less concordant (ICC = 0.488).
No significant difference (p = ns, de Long test) was detected among AUROC of the 3 techniques for predicting CSPH (figure).
We used the previously validated cut-off value of 21.1 kPa, to test the ability of each technique to correctly classify CSPH, obtaining variable accuracies in identifying correctly classified patients: 85% (34/40) for TE, 55% (17/31) for ElastPQ and 76% (19/25) for 2D-SWE, respectively.

Table: ROC analysis of each elastographic technique for predicting CSPH (HVPG >10 mmHg)

	AUROC	95% CI	p	Cut-off	Se	Sp
TE (n = 40)	0.872	0.761–0.983	<0.0001	21.5 kPa	0.75	1
ElastPQ (n = 31)	0.962	0.900–1.000	<0.0001	13.5 kPa	0.81	1
2D-SWE (n = 25)	0.860	0.715–1.000	<0.0001	19.7 kPa	0.72	1

Conclusions: Our findings showed that liver stiffness, assessed by new elastographic techniques (ElastPQ and 2D-SWE), have similar performance to predict CSPH like TE. However the best diagnostic threshold for this diagnosis differs significantly among different elastography technologies and specific diagnostic accuracy studies appear warranted for each type of equipment.

P0144
TWO TESTING STRATEGIES FOR COVERT HEPATIC ENCEPHALOPATHY DIAGNOSIS STABILIZES AGREEMENT BETWEEN SITES IN A MULTI-CENTER ANALYSIS: VALIDATION OF THE EASL/AASLD HE GUIDELINES

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Background and Aims: The recent EASL/AASLD guidelines suggest using ≥2 modalities are needed to diagnose covert HE in multi-center studies in order to reduce instability of the diagnosis between different modalities. However this approach has not been tested prospectively due to a lack of such studies in CHE. Aim: to compare whether agreement between sites is improved with using two abnormal strategies compared to a single strategy to diagnose covert HE.
Methods: Three sites [Virginia (VA), Ohio (OH) and Arkansas (AR)] enrolled outpatient cirrhotics (with/without treated overt HE) and age-matched healthy controls without chronic diseases. All subjects were given the psychometric hepatic encephalopathy score (PHES) and inhibitory control test (ICT) independently. Both tests were used by CHE independently by PHES or by ICT as gold standards compared to controls. Combined CHE was diagnosed if both PHES and ICT were abnormal. The rates of CHE diagnosis and analysis of agreement between centers and between each center and combined results using that cut point on either PHES/ICT or both were performed using Kappa statistics.
Results: We enrolled 302 healthy controls (103 VA, 100 OH, 105 AR) and 436 cirrhotic patients (230 VA, 107 OH, 99 AR). Sites were similar with respect to age (57±7 VA, 58±11 OH, 56±7 AR, p = 0.4) but VA patients had higher MELD score (12.5±6 VA, 10±4 OH, 10±3 AR, p < 0.0001) and frequency of prior overt HE (41% VA, 30% OH, 30% AR, p = 0.05). The combined rate of CHE diagnosis with ICT was

41% (30% VA, 50% OH, 53% AR) while for PHES the combined rate was 37% (45%VA, 30%OH and 25%AR). When both ICT and PHES were considered, 19% subjects had CHE overall (20% VA, 19% OH, 20% AR). Use of 2 abnormal tests stabilized the agreement between sites (Table).

Kappa statistics	ICT only abnormal	PHES only abnormal	Both abnormal
VA vs. OH	0.72	0.54	0.55
VA vs. AR	0.75	0.60	0.91
OH vs. AR	0.54	0.91	0.58
VA vs. combined	0.75	0.92	0.96
OH vs. combined	0.45	0.48	0.53
AR vs. combined	1.0	0.53	0.96

Conclusions: In this multi-center study, use of 2 abnormal testing strategies as proposed by the EASL/AASLD guidelines, increases agreement between sites and stabilizes the rate of CHE diagnosis. This approach can be used for developing/planning multi-center CHE trials.

P0145

PROSPECTIVE ANALYSIS OF FACTORS IMPLICATED IN EARLY READMISSIONS IN PATIENTS WITH DECOMPENSATED CIRRHOSIS

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Background and Aims: Early hospital readmissions (HR) in patients with decompensated cirrhosis (DC) increase health-care expenses and predict a poor outcome. The aim of this study was to identify the frequency and possible predictors of 30-day HR among patients with DC.
Methods: A total of 251 patients with DC discharged from 2 medical centers between October 2012 and September 2014 were followed. Patients with elective admissions or previous liver transplantation were excluded. Relationship between 30-day HR and multiple demographic, socioeconomic and clinical variables was studied by univariate and multivariate analyses.
Results: 75.3% were male, mean age was 62±12 years and 45% had previous admissions. Most frequent etiologies of cirrhosis were alcohol (66.5%) and hepatitis C (21.5%) and most common causes of admission ascites (29%), gastrointestinal bleeding (29%) and hepatic encephalopathy (HE) (17%). The median length of hospital stay was 7.7 days. Mean Child–Pugh and MELD scores and Charlson comorbidity index at discharge were 7.9±1.6, 13.3±5 and 4.3±1.8 respectively. 22% had an early HR (during the first 30 days after a hospital discharge). Univariate analyses showed that early HR were not significantly associated with sex (p = 0.3), age (p = 0.8), etiology of cirrhosis (p = 0.6), tobacco (p = 0.8), active alcohol use (p = 0.6), hepatocellular carcinoma (p = 0.9), history of previous admissions (p = 0.4) or level of education (p = 0.7). Early HR were significantly more frequent in patients who lived in urban areas (p = 0.05), not married (p = 0.04), admitted due to ascites or HE (p = 0.002), with Child–Pugh ≥10 (p < 0.001), MELD >15 (p < 0.001) or Charlson index >4 (p = 0.004) and in those with a length of hospital stay >12 days (p = 0.008). After adjusting for multiple covariates, independent predictors of early HR were admissions due to ascites or HE (OR 2.6 [95% CI: 1.3–5.1], p = 0.006), Child–Pugh ≥10 (OR 3.4 [95% CI: 1.6–7.2], p = 0.001), Charlson index >4 (OR 2.5 [95% CI: 1.2–5.3], p = 0.009) and a length of hospital stay >12 days (OR 3.1 [95% CI: 1.3–7.4], p = 0.008).
Conclusions: Almost 25% of patients with decompensated cirrhosis that required hospitalization have a HR within the first 30 days after discharge. The indication for index admission, severity of cirrhosis, presence of comorbidities and length of hospital stay are independent predictors of early HR. Conversely, socioeconomic factors do not seem to be related. It is necessary to develop and implement new strategies and interventions to prevent early re-hospitalization.

P0146

ORAL, ONCE-DAILY, CENICRIVIROC LEADS TO RAPID AND POTENT CCR2 AND CCR5 BLOCKADE IN SUBJECTS WITH ADVANCED LIVER DISEASE, SIMILAR TO EFFECTS SEEN IN HEALTHY VOLUNTEERS AND HIV-1 INFECTED SUBJECTS

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Background and Aims: Cenicriviroc (CVC), a potent, oral, once-daily CCR2/CCR5 antagonist, is in Phase 2b clinical development in adults with NASH and liver fibrosis, and is antifibrotic in animal models of liver and kidney disease, with favourable safety in ~580 subjects. CVC's antifibrotic activity is largely attributed to decreased monocyte/macrophage infiltration in the liver, leading to decreased hepatic stellate cell activation and reduced fibrogenesis. We aimed to characterise the immunologic effects of CVC across different populations.
Methods: Hepatic-impaired subjects (Child–Pugh class A or B; N = 15) and matched controls (N = 15) received CVC 150 mg daily for 2 weeks (Study 652–1–121). HIV-1-infected subjects (N = 52) re-

Table (abstract P0146).

Group	Baseline	Week 1		Week 2	
	Median, ng/L (IQR)	Median, ng/L (IQR)	Median fold change from BL	Median, ng/L (IQR)	Median fold change from BL
Cytokine MCP-1					
Mild hepatic impairment subjects (N = 7)	564.6 (539.9, 696.5)	2571 (2078, 4286)	5.1 (p = 0.02)	3483 (2193, 4170)	5.0 (p = 0.02)
Matched controls (N = 7)	571.8 (481.4, 762.7)	2520 (1971, 4688)	5.0 (p = 0.02)	3388 (2063, 5041)	5.9 (p = 0.02)
Moderate hepatic impairment subjects (N = 8)	631.8 (329.5, 956.7)	3026 (2405, 4584)	5.5 (p = 0.008)	3410 (2161, 4624)	6.0 (p = 0.008)
Matched controls (N = 8)	665.8 (509.1, 779.8)	2662 (2182, 4665)	4.4 (p = 0.008)	2902 (2256, 3920)	4.8 (p = 0.008)
HIV-1-infected subjects (N = 52)	196.2 (166.1, 253.2)	949.5 (773.3, 1091)	4.9 (p < 0.0001)	1059 (780.1, 1343)	5.3 (p < 0.0001)
Cytokine MIP-1β					
Mild hepatic impairment subjects (N = 7)	44.9 (31.7, 76.7)	82.6 (76.0, 97.6)	1.8 (p = 0.02)	89.0 (63.5, 96.9)	1.6 (p = 0.02)
Matched controls (N = 7)	41.5 (29.3, 52.5)	73.2 (58.9, 98.0)	1.9 (p = 0.02)	88.6 (72.2, 95.9)	2.5 (p = 0.02)
Moderate hepatic impairment subjects (N = 8)	37.2 (24.9, 66.4)	82.5 (63.8, 120.4)	2.3 (p = 0.008)	95.8 (57.5, 139.2)	2.5 (p = 0.008)
Matched controls (N = 8)	50.1 (42.9, 59.9)	103.6 (91.1, 137.1)	2.3 (p = 0.008)	108.3 (94.7, 152.4)	2.4 (p = 0.008)
HIV-1-infected subjects (N = 52)	43.5 (32.05, 57.6)	162.2 (121.5, 230.6)	3.9 (p < 0.0001)	176.0 (129.9, 249.3)	3.9 (p < 0.0001)

IQR, Interquartile range.

ceived CVC 200 mg daily with tenofovir/emtricitabine for ≤48 weeks (Study 652–2–202). MCP-1 (CCR2 ligand) and MIP-1β (CCR5 ligand) plasma levels were measured at baseline (BL) and after 1 and 2 weeks of CVC treatment in both studies; other CCR5 ligands (MIP-1α and RANTES), cytokines (IL-1β, IL-6, TNF-α) and microbial translocation markers (soluble CD14 [sCD14], LPS binding protein [LBP], intestinal fatty acid binding protein [I-FABP], flagellin) were measured in Study 121. Wilcoxon tests and Spearman correlations were used.
Results: Rapid and significant increases in MCP-1 and MIP-1β were observed after 1 and 2 weeks of treatment across all treatment groups (Table). In Study 121, as in Study 202, higher MCP-1 levels correlated with higher peak CVC concentration ($r = 0.39$, $p = 0.03$). In matched controls (moderate), TGF-β and RANTES increased during CVC treatment ($p = 0.008$, Week 2 vs BL). After 2 weeks of CVC treatment, there were no significant changes in MIP-1α, sCD14, LBP, I-FABP, flagellin, IL-1β, IL-6 or TNF-α. Flagellin correlated with I-FABP at BL ($r = 0.44$, $p = 0.01$) and Week 2 ($r = 0.40$, $p = 0.03$), and with ALT at BL ($r = 0.41$, $p = 0.02$) and Week 2 ($r = 0.45$, $p = 0.01$). In Study 202, sustained reductions in sCD14 were observed over 48-week CVC treatment ($p < 0.001$, CVC vs efavirenz).
Conclusions: CVC treatment led to rapid, reciprocal increases of MCP-1 and MIP-1β, due to effective CCR2 and CCR5 blockade across all treatment groups, regardless of underlying pathology; the greater magnitude in MIP-1β changes in HIV-1-infected subjects could be attributed to HIV binding to CCR5. Correlation between MCP-1 and CVC concentrations suggests a dose-dependent effect. Importantly, increased MCP-1 and MIP-1β levels were not associated with increased markers of intestinal damage, or hepatic or systemic inflammation.

P0147
MALNUTRITION AND ADHERENCE TO NUTRITIONAL RECOMMENDATION IN PATIENTS WITH CIRRHOSIS

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Background and Aims: Malnutrition proved to be prevalent in patients with cirrhosis and has prognostic relevance. The aim of our study was to: check the prevalence of malnutrition in a population with decompensated cirrhosis and to evaluate the adherence to nutritional recommendations.
Methods: 101 consecutive patients with cirrhosis (64 hospitalized for decompensation and 37 compensated from outpatient ward) were prospectively evaluated for the presence of malnutrition using Subjective Global Assessment (SGA) criteria and mid-arm circumference (MAC). Malnutrition was defined as SGA class B and C and MAC <10th. All patients were interviewed regarding their food intake using an adapted questionnaire and the energy and main nutrients (proteins, lipids, carbohydrates and salt) intake was calculated.
Results: According to the SGA, in the decompensated group 48 patients (75%) had malnutrition, while only 9 patients (24%) were malnourished in the compensated group. Using the MAC criteria, malnutrition was present in 41 (64%) decompensated and 13 (35%) compensated patients. Only 13 (12.9%) patients had the recommended alimentary intake, 78 (77.2%) patients had a suboptimal energy intake and only 26 (25.7%) patients had the recommended protein intake. Only 26 (25.7%) among all and 16 (25%) among decompensated patients were adherent to low salt diet.
Lower cholesterol levels (HR = 0.97, $p = 0.001$), lower sodium (HR = 0.71, $p = 0.004$) and female sex (HR = 8.25, $p = 0.01$) are independently associated with presence of malnutrition.
Conclusions: Prevalence of malnutrition is high in patients with advanced cirrhosis and is related in part to a low adherence to nutritional recommendations.

P0149
SIX-MONTH MORTALITY OF CIRRHOTIC PATIENTS WHO SURVIVED INTENSIVE CARE: A META-ANALYSIS

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Background and Aims: The medium-term survival of cirrhotic patients who survived intensive care and its determinants have never been evaluated due to the small number of ICU survivors in the published studies. This meta-analysis evaluated the predictors of 6-month mortality in ICU survivors.
Methods: 13 studies (2695 cirrhotics) were analyzed after selection of original articles and response to a standardized questionnaire by the corresponding authors. The endpoint was 6-month mortality of ICU survivors. 95 pooled analyses concerned patient characteristics, reason for admission, organ replacement therapy, and composite scores.
Results: 5 studies reported the outcome of 412 ICU survivors. Only 48 patients (3.4%) were transplanted during follow-up. Six-month mortality was lower in high volume centers (OR = 0.45;95% CI:0.30–0.67;$p < 0.001$), in general ICUs (OR = 0.31;95% CI:0.21–0.47;$p < 0.001$) in centers with TIPS (OR = 0.42;95% CI:0.25–0.46;$p = 0.002$), but not with liver transplantation (OR = 2.21;95% CI:1.21–4.04;$p = 0.008$) available. Age, sex and alcohol-related cirrhosis had no significant impact on 6-month mortality. Unlike for in-ICU mortality, high values of SOFA did not predict 6-month mortality in ICU survivors. Eight parameters of liver and renal function were associated with 6-month mortality, including Child–Pugh C stage (OR = 2.43;95% CI:1.44–4.10;$p < 0.001$), MELD ≥26 on ICU admission (OR = 3.97;95% CI:1.92–8.22;$p < 0.0001$;PPV = 0.75), hepatorenal syndrome (OR = 4.67;95% CI:1.24–17.64;$p = 0.022$;PPV = 0.88) and admission for acute renal failure (OR = 3.29;95% CI:1.70–6.40;$p < 0.001$;PPV = 0.73). Septic shock (OR = 3.95;95% CI:1.38–11.30;$p = 0.010$;PPV = 0.62) and nosocomial infection on admission (OR = 2.72;95% CI:1.09–6.76;$p = 0.031$;PPV = 0.76) were also associated with higher 6-month mortality. Among medical interventions, only the use of norepinephrine (OR = 2.07;95% CI:1.07–4.00;$p = 0.029$;PPV = 0.61), given for hepatorenal syndrome, was predictive of 6-month mortality in ICU survivors.
Conclusions: Only a minority of ICU survivors undergo liver transplantation. Liver and renal failures in ICU have a sustained impact on long-term mortality. The prognostic performance of general ICU scores decreases over time, unlike Child–Pugh and MELD scores, even measured in the context of organ failure. Eligible patients could thus be listed for transplantation in ICUs or shortly after ICU discharge.

P0150
LIVER CIRRHOSIS IS INDEPENDENTLY ASSOCIATED WITH MORTALITY IN PATIENTS WITH BACTERIAL ENDOCARDITIS: RESULTS OF A CASE CONTROL MULTICENTER STUDY OF 202 CASES

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Background and Aims: Bacterial endocarditis (BE) is severe in patients (pts) with liver cirrhosis (LC); data rely on uncontrolled studies. Aims of this study were to compare clinical presentation and mortality of BE in LC pts with those of controls (CT) without cirrhosis matched for sex, age and diabetes.
Methods: Medical charts for all LC pts with BE seen in 23 liver units (2000–2013) were reviewed and compared with those of CT. Short-term mortality was analyzed by univariate and logistic regression analysis.
Results: 101 BE in LC pts and 101 BE in CT were analyzed. LC pts: 63.3 [42–87] years, CT: 65 [46–93]; 145 M (72%). LC causes: alcohol: 78 (67.2%), viral: 17 (14.6%), NAFLD: 14 (12%). Child–Pugh scores: A: 8.8% pts, B: 42.9% pts, C: 48.3%. In LC pts: total bilirubin was 67.8 μmol/l±77.7, prothrombin time (PT) 52.7±18.1%, serum albumin 25 g/l±5.9, serum creatinine (SC) 123.5 μmol/l±103.6. Blood cultures were positive in 181 pts (92%). 39 LC pts (40.2%) and 52 CT (53.6%) had a pre-existing heart disease (NS). At diagnosis, 43 LC (47.7%) and 31 CT (34.0%) exhibited heart failure (NS). Severe sepsis or septic shock was noted in 33 LC pts (33.3%) and 23 CT (23.0%) (NS). The source of contamination was digestive, urinary, or cutaneous (DUC) in 41 LC pts and in 21 CT (OR 2.4 [1.1–5.1], p < 0.01). The aortic valve was involved in 68.4% of LC pts and 47.7% CT (NS). The isolated bacteria (Staphylococcus n = 67: LC = 33, CT = 34; Streptococcus n = 92: LC = 48, CT = 44) were comparable as well as antibiotics and the duration of treatment. 26 LC (27.1%) and 44 CT (44.9%) had heart surgery (0.5 [0.2–0.9], p < 0.01). 56 LC (62.2%) and 25 CT (27.8%) died (OR: 6.1 [3–12.5], p < 0.0001). By univariate analysis death was related in CT pts with presence of severe sepsis or shock (p = 0.02). In LC pts mortality was related with PT <40% (OR 6.75 [1.6–32.7], p = 0.002), SC value (p < 0.03), ascites (OR 7.4 [1.7–34.2], p = 0.001) and total bilirubin (p = 0.02). By multivariate analysis, mortality was related in overall population with cirrhosis: (OR 2.35 [1.04–5.36], p = 0.04) and PT <40% (OR 7.52 [2.41–29.6], p < 0.001). In LC pts, mortality was related with PT <40% (OR 21.6 [2.9–508], p = 0.01) and ascites (OR 8.3 [1.4–69], p = 0.03).
Conclusions: Bacterial endocarditis is very severe in LC pts with less access to surgery. DUC sources of contamination are more frequent in LC pts than in controls. Cirrhosis is independently associated with mortality with and increased risk of death by 2.35. Predictors of death in cirrhotic patients are a PT <40% and the presence of ascites.

P0151
COVERT HEPATIC ENCEPHALOPATHY COMPRISES TWO CLINICALLY AND PATHOPHYSIOLOGICALLY DISTINCT SYNDROMES: A PROSPECTIVE LONGITUDINAL STUDY

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Background and Aims: EASL/AASLD hepatic encephalopathy (HE) guidelines proposed that Grade 1 HE and minimal HE (mHE) be combined into a single entity referred to as 'Covert HE'. However, prospective, longitudinal data to indicate that these are indeed a single entity are lacking. The study aims were to determine whether these entities were clinically and pathophysiologically similar. The end-points were (a) occurrence of complications of cirrhosis such as variceal bleeding, infection, ascites and HE requiring hospital admission and mortality (b) ammonia levels and inflammatory markers and (c) neutrophil function.
Methods: Clinically-stable liver cirrhosis out-patients (n = 106; age 58±10 years; 67 men; aetiology: viral: 44, alcohol: 43) with no previous history of HE were included over a 2-year period and classified as having no HE (n = 23), mHE (n = 39) or Grade 1 HE (n = 44) using the West-Haven criteria, EEG, psychometric tests (PHES) and critical flicker frequency (CFF). Patients were followed clinically and biochemically for a median of 241±110/234±105/229±93 days. Venous ammonia, bacterial DNA and neutrophil respiratory burst were measured at inclusion and the patients were followed for the occurrence of complications requiring hospitalisation and death.
Results: Patients with Grade 1 HE had more complications (infection 9/18/34%; HE 4/8/18%; other 9/13/14%; P = 0.02) and had significantly greater mortality (9/8/23%; P = 0.02) compared to patients with no HE or mHE respectively. Ammonia and white cell count were not different between patients with mHE and Grade 1 HE but ammonia was higher than no HE group (48±11/61±14/62±12 μmol/L; P < 0.001). Severity of liver disease was similar in the 3 groups (MELD score: no HE: 15±5, mHE: 13±6, Gr 1 HE: 16±6). However, patients with Grade 1 HE had increased bacterial translocation (57% vs. 36%; P = 0.02) and neutrophil spontaneous respiratory burst (22±22% vs. 13±14%; P = 0.02) compared to patients with mHE indicating susceptibility to infection in these patients.
Conclusions: The results of this study show for the first time that 'Covert HE' is a heterogeneous entity comprising two clinically and pathophysiologically distinct syndromes. The most common complication necessitating hospital admission in Grade 1 HE patients is infection and this is associated with bacterial translocation and neutrophil dysfunction. Future studies should address this novel finding of HE induced susceptibility to infection.

P0152

THE EFFECT OF IRSOGLADINE MALEATE FOR PORTAL HYPERTENSIVE GASTROPATHY (PHG)

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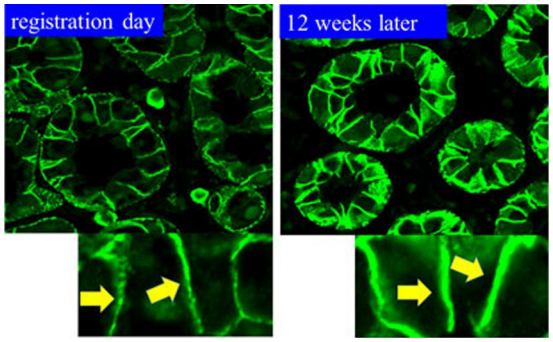
Background and Aims: Portal hypertensive gastropathy (PHG) due to portal hypertension (PH) is characterized by noninflammatory edema and vasodilatation of the lamina propria of mucous membrane. In addition, the decrease in intercellular junctional proteins and the dilatation of the endothelium gap is also said to be one of causes. Therefore, some relationship between PHG and tight junction (TJ) which is one of intercellular adhesions has been suggested. In this study, we examined the possibility that Irsogladine maleate would improve PHG by having restoration of TJ.
Methods: Eighteen patients with PHG were registered and randomly assigned in a ratio of 2:1 the irsogladine maleate administration group and the non-administration group. In the administration group, irsogladine maleate (4 mg/day) was administered orally from the registration day until 12 weeks later. Redness mucosas of the PHG were collected endoscopically at the registration day and 12 weeks later and compared. Evaluation methods for therapeutic effect determination were itemized as follows, (a) macroscopic findings by endoscope, (b) Immunological findings of claudin-3 protein (TJ protein) by laser microscope in gastric mucosal tissue, (c) quantification of claudin-3 protein (Western blotting) in gastric mucosal tissue.
Results: The macroscopic findings were improved in two of 12 patients in the administration group, and the laser microscope image which suggested restoration of TJ by immunological findings provided in eight patients. In addition, in a total of 21 samples in 12 patients of the administration group and a total of 12 samples in 6 patients of the non-administration group, the percentage of samples to increase in the claudin-3/β-actin (protein quantification/control) after 12 weeks was larger in the administration group than the non-administration group (66.7% vs 33.3%). Moreover in claudin-3/β-actin, the average value subtracted registration day value from 12 weeks later value in the administration group was significantly higher than the non-administration group ($p = 0.0386$). Therefore, it showed that higher effect of TJ restoration in the administration group.
Conclusions: In this study, we suggested the possibility that irsogladine maleate would prevent progression of PHG and bleeding by raising the ability for restoration of TJ failure.



P0153

INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN 3 (IGFBP3) SERUM LEVELS ARE ASSOCIATED WITH PROGNOSIS IN PATIENTS ADMITTED FOR ACUTE DECOMPENSATION OF CIRRHOSIS

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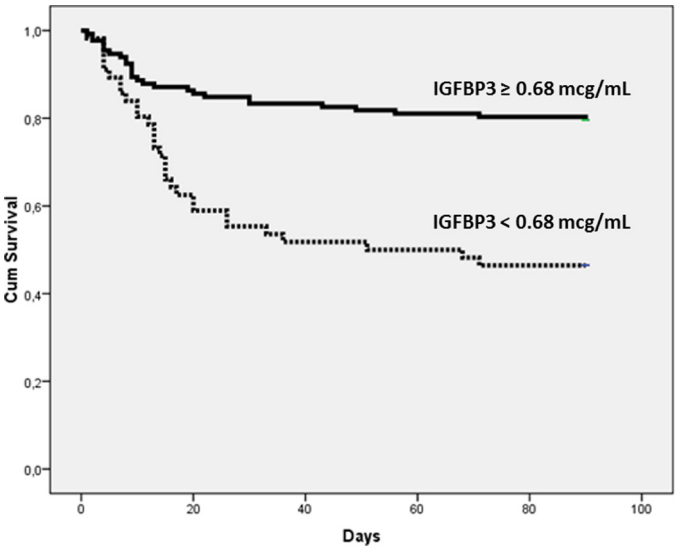
Background and Aims: Decreased IGFBP3 serum levels have been reported in patients with cirrhosis and seem to correlate with hepatic dysfunction intensity. However, data about its prognostic significance is still lacking. We sought to investigate the relationship between serum IGFBP3 levels and short-term prognosis in patients admitted for acute decompensation of cirrhosis.
Methods: In this prospective cohort study, patients admitted in the emergency department were followed during their hospital stay and 90-day mortality was evaluated by phone call. ACLF criteria were applied according to the EASL-CLIF Consortium definition. Twenty-nine patients were also evaluated in the outpatient clinic after discharge and were compared in two moments (inpatient and outpatient evaluation).
Results: Between January 2011 and November 2013, 189 patients were included, with a mean age of 53.6±11.6 years, and a male predominance (73.0%). The mean MELD score was 16.3±6.5 and 39.7% of the individuals were Child–Pugh C. IGFBP3 levels positively correlated with sodium and albumin levels and negatively correlated with INR, CPR, total bilirubin and MELD score. Significantly lower IGF-1 levels were observed in Child–Pugh C ($P < 0.001$) and in ACLF ($P = 0.007$) patients. The 90-day mortality was 30.3% and it was associated in the bivariate analysis with active alcoholism, bacterial infection, ascites, hepatic encephalopathy, Child–Pugh C and ACLF at admission. Concerning laboratory data, 90-day mortality was associated with higher leucocyte count, creatinine, INR, CRP, venous lactate, total bilirubin, MELD and lower sodium, albumin and IGFBP3 at admission. Logistic regression analysis including variables with $P < 0.01$ in the bivariate analysis showed that MELD score, ascites at admission and IGFBP3 <0.68 mcg/mL were independently associated with 90-day mortality. The Kaplan–Meier survival probability (Figure) at 90-day was 79.5% in patients with IGFBP3 ≥0.68 mcg/mL and 46.4% for subjects with IGFBP3 <0.68 mcg/mL ($P < 0.001$). Significantly lower IGFBP3 levels were found at the time of acute decompensation of cirrhosis as compared to the outpatient evaluation (1.19±0.57 mcg/mL vs. 1.69±0.86 mcg/mL, $P = 0.001$).



Conclusions: IGFBP3 levels correlated with the severity of hepatic dysfunction and decrease during acute decompensation of cirrhosis. IGFBP3 was also independently associated with short-term mortality and thus may represent a promising tool in assessing prognosis of cirrhosis.

P0154

DIFFERENTIAL LEUKOCYTE COUNT AS AN ADJUNCTIVE BIOMARKER OF OUTCOME IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE

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Background and Aims: Susceptibility to bacterial infection and activation of systemic inflammatory responses (SIRS) are poor prognostic features in acute decompensation (AD) of cirrhosis and acute-on-chronic liver failure (ACLF). In non-hepatic systemic inflammatory disorders, circulating neutrophil to lymphocyte ratios are correlated with infectious complications and mortality. Peripheral blood leukocyte concentrations are not part of modern systems for outcome prediction such as the chronic liver failure sequential organ failure assessment (CLIF-SOFA). We determine the prognostic utility of differential leukocyte concentrations in patients with AD and ACLF.

Methods: 51 patients with ACLF were consecutively recruited. Peripheral blood leukocyte concentrations were measured on hospital or liver intensive care admission and correlated to MELD, CLIF-SOFA score, culture positive bacterial infection (CPBI) and hospital survival. A further 243 patients with ACLF and 67 patients with AD were used as a validation set.

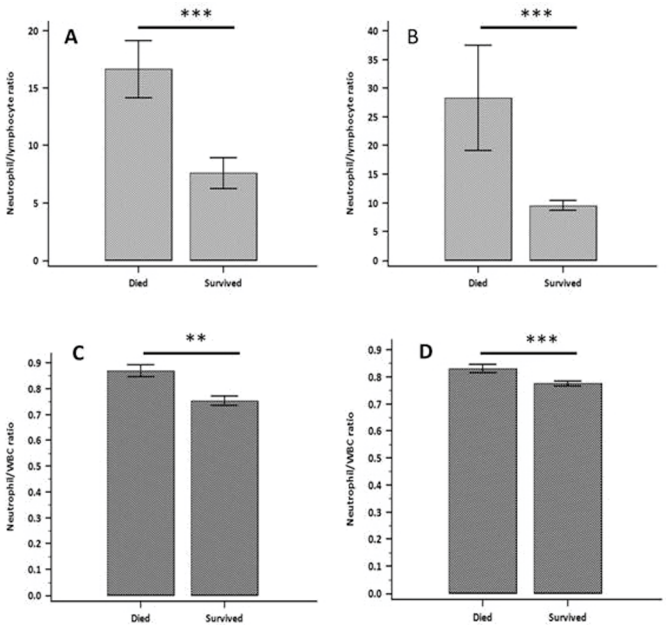

Figure 1. Mean (SEM) values of neutrophil/lymphocyte ratio and neutrophil/white blood cell (WBC) ratio in the derivation (A,B) and validation (C,D) ACLF cohorts (**p < 0.001; ***p < 0.0001).

Results: Total white cell count was not different between survivors or non-survivors. Non-survivors had higher neutrophil concentrations and lower lymphocyte concentrations. Both neutrophil/lymphocyte concentration ratio (NLR; p < 0.0001) and neutrophil/WBC ratio (NWCR; p < 0.001) were higher in non-survivors as was monocyte lymphocyte ratio (MLR). SIRS score, NLR and NWCR were not dependent on the presence of CPBI. Both NLR and NWCR correlated with CLIF-SOFA (r = 0.647, p < 0.001 and r = 0.619, p < 0.001). NLR AND NWCR predicted outcome with AUROC of 0.802 (95% CI 0.677–0.927) and 0.821 (95% CI 0.704–0.938) respectively. In the validation cohort NLR and NWCR were again higher in non-survivors (p < 0.0001 for both). Only MLR was predictive of CPBI [AUROC 0.706 (0.563–0.848), p = 0.004, cut-off >0.438]. Addition of NLR to SOFA score gave higher AUROCs (0.768 v 0.731, p = 0.03) but did not improve accuracy of CLIF-SOFA. In the AD validation set NLR predicted outcome but with a reduced accuracy [AUROC 0.681 (0.532–0.830), p = 0.017].

Conclusions: ACLF is associated with high neutrophil and low lymphocyte counts, reflecting activation of SIRS responses. NLR and NLWC are associated with higher organ failure scores and mortality rates. NLR improves the outcome predictive accuracy of SOFA but not CLIF-SOFA. NLR and NWCR are associated with multi-organ failure whereas MLR predicts the presence of BI. These parameters may be incorporated into established prognostic scoring systems for earlier prediction of bacterial infections.

P0155

VALIDITY AND RELIABILITY OF SPLENIC VOLUME MEASUREMENT BY ULTRASOUND AND ITS CLINICAL IMPLICATIONS TO DEFINE SPLENOMEGALY IN LIVER CIRRHOSIS

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Background and Aims: Presentation of splenomegaly is a typical sign of liver cirrhosis. However, there are few studies for establishing the definition of splenomegaly. In this study, we aimed to measure splenic volume (SV) using ultrasound (US) and compared with computed tomography (CT) image as a reference standard and also try to find out its relationship with portal hypertension and best cut-off value for discriminating cirrhosis from non-cirrhosis.

Methods: SV was measured with US in 1,256 patients with chronic liver disease from August 2007 to March 2014. SV was obtained by conventional prolated ellipsoid formula (0.524×length×width×height). We evaluated the reliability of SV measurement which was performed by two sonographers simultaneously from randomly selected 82 patients (164 cases) and assessed the validity by the source image of CT from 105 patients. Liver biopsy was done in 217 patients within 6 month from SV measurement. We also evaluated the relationship between liver fibrosis and hepatic venous pressure gradient (HVPG) and SV. We did a sensitivity analysis with SV adjusted by body surface area (BSA).

Results: The concordance correlation coefficient (CCC) between two sonographers was 0.9886 (95% confidence interval [CI], 0.9816–0.9930) and CCC between the measurement by US and CT was 0.9721 (95% CI, 0.9556–0.9826). SV was increased in male, young ages and significantly larger in viral liver cirrhosis than alcoholic liver cirrhosis. SV showed a positive correlation with Child–Pugh score, MELD score and was increased greatly in patients having esophageal varices, gastric varices or ascites compared to those who had not. There was a significant difference of SV between fibroscan score (≤12 versus >12 kPa) in viral liver cirrhosis, but not in alcoholic liver cirrhosis. The AUROC of SV for the prediction of F4 was 0.812 (95% CI, 0.749–0.865, p < 0.001) and the optimal cut-off value of SV was 300.3 cm^3. SV/BSA also showed a good prediction for F4 (AUC, 0.802; 95% CI, 0.736–0.857; p < 0.001). However, there was no significance of SV for predicting significant HVPG (≥12 mmHg) (AUC, 0.578; 95% CI, 0.499–0.654; p = 0.106).

Conclusions: Splenic volume measured by US is precise and accurate and highly associated with the incidence of liver cirrhosis complications. Splenic volume about 300 cm^3 was an optimal cut-off value to discriminate between cirrhosis and non-cirrhosis.

P0156

RISK FACTORS ASSOCIATED WITH OVERALL AND BLEEDING-RELATED MORTALITY IN PATIENTS WITH PORTAL VEIN THROMBOSIS ON THE WAITING LIST FOR LIVER TRANSPLANTATION

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Background and Aims: The reported prevalence of PVT is increasing in patients with ESLD awaiting LT. It significantly affects waiting list survival, complicates the liver transplant operation and impacts post-transplant survival and morbidity. Anticoagulation is a challenging therapy in patients with ESLD because of the well-recognized coagulation abnormalities in cirrhotics, the increased risk of bleeding, and the lack of evidence of a real clinical benefit from the therapy. The aim of the study was to investigate the risk factors for overall and hemorrhage related death in a cohort of 104 cirrhotic patients with PVT included on the waiting list for LT.
Methods: We tested separately the association between different parameters and overall death while on the waiting list using Cox regression model.
Results: There were 68.3% men with a mean age of 53.0±9.8 years, 30.8% of patients had HCV and 36.5% had HBV-related cirrhosis. Overall death was encountered in 31.7% of patients, out of whom 14 (13.5%) were hemorrhage-related. As independent risk factors for overall death were identified the following: associated superior mesenteric vein thrombosis ($p = 0.04$), refractory ascites with frequent paracentesis ($p = 0.01$), shorter time from liver cirrhosis to PVT diagnosis ($p < 0.0001$). Hemorrhage-related death was associated only with the administration of anticoagulation therapy ($p = 0.002$).
Conclusions: Anticoagulant therapy is associated with higher risk of hemorrhage-related death, but not with overall death on the waiting list.

P0157

ADHERENCE AND SAFETY OF BETA-BLOCKERS IN ELDERLY PATIENTS WITH CIRRHOSIS AND PORTAL HYPERTENSION: A MULTI-CENTER CROSS-SECTIONAL STUDY

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Background and Aims: In elderly patients co-morbidities are frequent and may affect therapy because of drug interactions. In patients with cirrhosis non-selective beta-blockers (NSBBs) can be given as a prophylaxis of variceal bleeding. The best protective effect is obtained with drug doses sufficient to stably reduce the heart rate below 60 bpm. However, the adherence to such a schedule in old cirrhotic patients with co-morbidities has never been investigated. The aim of the present study was to assess the adherence and safety of BBs in >65 years old in-patients with cirrhosis and co-morbidities.
Methods: One-hundred eighty-four cirrhotic patients admitted in 38 Italian internal medicine wards were prospectively studied during a period of 4 weeks. Their clinical and pharmacological history, the length of hospital stay (LOS), and the occurrence of adverse events were recorded and tested by uni- and multi-variable analysis to identify possible associations with the use of BBs.
Results: At admission 64 of 184 cirrhotic patients (35%) were receiving BBs. Thirty-two were receiving a NSBB and in 26 the main indication was the prophylaxis of portal hypertension related bleeding. In only 5 of these patients heart rate was <60 bpm. LOS was 8.6±6.3 days for cirrhotic patients not-receiving BBs, 10.4±6.3 for patients receiving BBs for portal hypertension, and 13.8±12.2 for patients receiving BBs for cardio-vascular disease ($p = 0.016$). Twenty-five patients had kidney failure associated with the use of BBs ($p < 0.01$). However, this association was lost after adjusting for other factors significantly associated to kidney failure. At multivariable analysis, only older age and PPI use were independently associated with kidney failure.
Conclusions: The doses of BBs given as a prophylaxis of bleeding in most elderly in-patients with cirrhosis and co-morbidities are probably insufficient being the patient HR >60 bpm in 80% of cases. The use of BBs in such patients is associated with longer LOS and kidney failure. However, a cause-effect relationship between BBs and renal function was excluded in our series. A better appropriateness in the BBs prescription should be recommended in elderly cirrhotic patients with portal hypertension.

P0158

INFLUENCE OF SUSTAINED VIRAL RESPONSE IN THE REGRESSION OF FIBROSIS AND PORTAL HYPERTENSION IN CIRRHOTIC HCV GENOTYPE 1 PATIENTS TREATED WITH TRIPLE THERAPY

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Background and Aims: Treatment with protease inhibitors has allowed a SVR above 50% in HCV genotype 1 cirrhotic patients. However, regression of liver fibrosis and portal hypertension (PH) has not been studied. Our objective was to evaluate the influence of SVR in portal pressure gradient (HVPG), and non-invasive parameters of PH and fibrosis by Fibroscan®. Also, predictors of SVR were studied.
Methods: From November 2012 to August 2013, 16 compensated HCV genotype 1 cirrhotic patients with PH (HVPG >6 mmHg), without beta-blocker therapy, candidates to PEGα2a+RBV+BOC (48 weeks, lead-in). An hemodynamic study and a FibroScan® were performed at baseline, 8 weeks and, in the case of SVR, 24 weeks after treatment. In each hemodynamic study serum samples of inflammatory biomarkers associated with PH were analyzed (VCAM1, IL-1β and IL-1Rα) and renin/aldosterone. Statistical analysis was performed using the SPSSv20 program.
Results: Baseline characteristics: age 55±8years, 6 males (37%), previous response (naive n = 10; n = 5 null response; relapsers n = 1), 130±58*103platelets/L, albumin 4±0.4 mg/dl, bilirubin 0.9±0.4 mg/dL, ALT 61±6 IU/L, renin 7.4±4 ng /mL, aldosterone 62.9±51.7 ng/mL, Child–Pugh 5±0.5, MELD 6.6±1, Fibroscan®21.3±14.5 kPa, HVPG 10.6±4.3 mmHg, esophagueal varices (small n = 2; large n = 2). 8 patients achieved SVR, 5 relapsed, and treatment was stopped due to non-response to lead-in (n = 1) and decrease <3 log viral load at week 8 (n = 2). Compared to baseline, at weeks 8 and 72 there was a significant decrease in HVPG (10.6±4.3 vs 9.4±5.04 vs 6.1±3.61 mmHg, $p < 0.0001$) and Fibroscan® values (21.3±14.5 vs 16.2±9.5 vs 6.4±4.5 kPa, $p < 0.0001$). In the multivariate analysis, factors predicting SVR were a prior null response ($p = 0.042$) and renin baseline value ($p = 0.038$). The HVPG average decrease in SVR was 38.5±18%, achieving a HVPG <6 mmHg in 5 patients (62.5%) and Fibroscan® <7.1 kPa in n = 3 (37.5%). There was also a significant decrease at weeks 8 and 72 in VCAM1, IL-1β and IL-1Rα values ($p < 0.0001$) with a strong positive

correlation with Renin/Angiotensine axis r = 0.875 p = 0.016 in SVR patients.
Conclusions: Complete hemodynamic response (HVPG <6 mmHg) and fibrosis regression (Fibroscan® <7.1 kPa) were observed in more than half and one third of SVR patients, respectively. We strongly suggest that HVPG and fibrosis response must be evaluated in SVR-cirrhotic patients due to their potential influence on risk of liver decompensation and hepatocellular carcinoma. The new direct antiviral agents should also be evaluated in these terms.

P0159
NON INVASIVE BLOOD FLOW MEASUREMENT BY "ARTERIAL SPIN LABELING" DETECTS MINIMAL HEPATIC ENCEPHALOPATHY

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Background and Aims: To assess whether non invasive blood flow measurement by arterial spin labeling in several brain regions detects minimal hepatic encephalopathy
Methods: Blood flow (BF) was analyzed by arterial spin labeling (ASL) in different brain areas of 14 controls, 24 cirrhotic patients without and 16 cirrhotic patients with minimal hepatic encephalopathy (MHE).
Images were collected using a 3 Tesla MR scanner (Achieva 3T-TX, Philips, Netherlands). Pulsed ASL was performed. Patients showing MHE were detected using the battery Psychometric Hepatic Encephalopathy Score (PHES) consisting of five tests. Different cognitive and motor functions were also assessed: alterations in selective attention were evaluated using the Stroop test. Patients and controls also performed visuo-motor and bimanual coordination tests. Several biochemical parameters were measured: serum pro-inflammatory interleukins (IL-6 and IL-18), 3-nitrotyrosine, cGMP and nitrates+nitrites in plasma, and blood ammonia. Bivariate correlations were evaluated.
Results: In patients with MHE, BF was increased in cerebellar hemisphere (P = 0.03) and vermis (P = 0.012) and reduced in occipital lobe (P = 0.017). BF in cerebellar hemisphere was also increased in patients without MHE (P = 0.02). Bimanual coordination was impaired in patients without MHE (P = 0.05) and much more in patients with MHE (P < 0.0001). Visuo-motor coordination was impaired only in patients with MHE (P < 0.0001). Attention was slightly affected in patients without MHE and more strongly in patients with MHE (P < 0.0001). BF in cerebellar hemisphere and vermis correlated with performance in most tests of PHES [number connection tests A (NCT-A), B (NCT-B) and line tracing test] and in the congruent task of Stroop test. BF in frontal lobe correlated with NCT-A. Performance in bimanual and visuomotor coordination tests correlated only with BF in cerebellar hemisphere. BF in occipital lobe correlates with performance in the PHES battery and with CFF. BF in cerebellar hemisphere correlates with plasma cGMP and nitric oxide (NO) metabolites. BF in vermis cerebellar also correlates with NO metabolites and with 3-nitrotyrosine. IL-18 in plasma correlates with BF in thalamus and occipital lobe.
Conclusions: Non invasive BF determination in cerebellum using ASL may detect MHE earlier than the PHES. Altered NO-cGMP pathway seems to be associated to altered BF in cerebellum.

P0160
LIVER CIRRHOSIS IN SOUTHERN SWEDEN 2001–2011: A POPULATION-BASED STUDY

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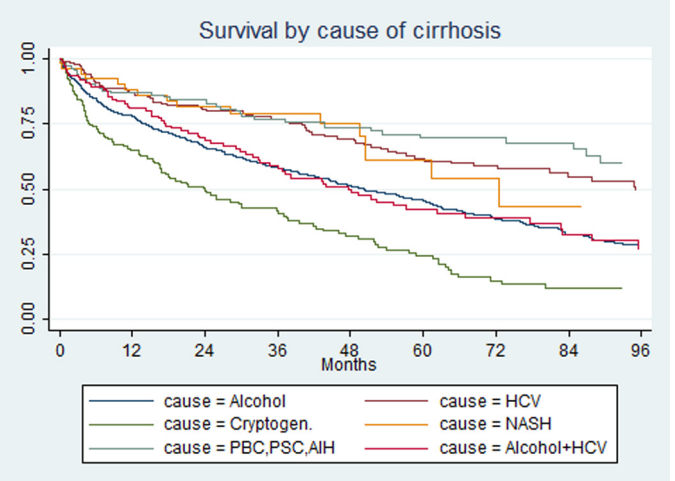
Background and Aims: In Sweden, the most common causes of liver cirrhosis are alcohol overconsumption and hepatitis C. While epidemiological data suggest that Sweden has a low cirrhosis-related mortality compared with other European countries, recent data on the incidence, prevalence and outcome of cirrhosis are scarce. We undertook a systematic, population-based study of liver cirrhosis in southern Sweden.
Methods: We carried out a retrospective study in Skåne county, the southernmost part of Sweden. The region has a population of 1,169,464 (year 2005) and has a single-provider system for hospital care. Using population-based diagnosis registries, we identified 4,210 patients with liver disease (ICD-10 codes K70.3, K74.6, K74.3, B18.2G, B18.1G, K76.0, K83.0, K73.2, K75.4, I.85.0, I85.9 and C22.9). Following review of medical records and histopathology data, we identified and included 1,263 patients who were diagnosed with cirrhosis between January 2001 and December 2010. The aetiology was classified as alcohol-induced, HCV, cryptogenic, NASH, PBC, PSC, AIH, or other. Clinical parameters were registered, including age at diagnosis, complications at diagnosis, and date of death. All patients were followed until death or December 2011. The median observation time was 4.8 years for surviving patients. The median age at diagnosis was 60 years.
Results: The crude annual incidence of cirrhosis in southern Sweden was estimated at 14.8/100,000, 9.8 for women and 20.1 for men. No changes in incidence were observed during the study period. The most common aetiology was alcohol overconsumption (57%) followed by HCV (13%) and cryptogenic cirrhosis (11%). The most common complications at diagnosis were ascites (43%), variceal bleeding (6%) and overt encephalopathy (4%). The total one- and five-year survival rates were 79% and 48%, respectively. In patients with complications at diagnosis these survival rates were 69% and 37%. Patients with cryptogenic cirrhosis showed the highest mortality followed by alcoholic cirrhosis with or without concomitant HCV (Figure 1).
Conclusions: We present the largest population-based study describing baseline cirrhosis epidemiology in Sweden to date. Our results support that the incidence of cirrhosis in Sweden is comparable with that observed in other European countries. Mortality varies with aetiology. Notably, cirrhosis on the basis of HCV and alcohol in combination showed as poor outcome as alcoholic cirrhosis alone.



P0161

INCIDENCE AND RISK FACTORS FOR FALLS IN PATIENTS WITH COMPENSATED CIRRHOSIS

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Background and Aims: Falls are frequent in patients with decompensated cirrhosis, particularly in those with subclinical cognitive dysfunction (CD). However, the incidence and risk factors for falls in patients with compensated cirrhosis are not known. The aims were to analyze the difference in the incidence of falls between patients with compensated cirrhosis and those with decompensated cirrhosis, and to evaluate risk factors for falls in the two groups.
Methods: We studied a cohort of outpatients with compensated or decompensated cirrhosis. The presence of CD was evaluated by the Psychometric Hepatic Encephalopathy Score (PHES). Patients were considered to have CD when their PHES was <−4. We prospectively recorded the incidence of falls during follow-up by evaluation every three months. The predictive factors associated with falls were determined by Cox regression analysis, and the probability of falls was estimated by Kaplan–Meier method.
Results: We included 302 patients, 189 men and 113 women. Mean age was 63.0±10.8 years and 83/302 (27.4%) had CD. Mean follow-up was 28.8±18.5 months. We found 32/122 (26.2%) patients with compensated cirrhosis fell during follow-up, compared to 49/180 patients (27.2%) with decompensated cirrhosis ($p = 0.89$). The independent predictive factors of falls in the multivariate analysis were CD (HR 2.7, 95% CI 1.2–6.2, $p = 0.01$) and age (HR 1.065, 95% CI 1.024–1.108, $p = 0.002$) in compensated patients; and CD (HR 3.0, 95% CI 1.6–5.6, $p < 0.001$), previous falls (HR 2.4, 95% CI 1.3–4.5, $p < 0.007$), viral etiology of cirrhosis (HR 2.3, 95% CI 1.2–4.3, $p = 0.008$) and comorbidity degree (HR 1.323, 95% CI 1.123–1.558, $p = 0.001$) in decompensated patients. The 2-year probability of falls was 24.8% in compensated patients: 63.0% in patients with CD and 18.7% in patients without CD ($p < 0.001$); and 23.8% in decompensated patients ($p = 0.38$ with respect to compensated patients): 50.3% in patients with CD and 12.0% in patients without CD ($p < 0.001$). Injuries caused by the falls were more severe in decompensated patients than in compensated patients ($p = 0.01$), but no differences were found between patients with and without CD.
Conclusions: The risk of falling in patients with compensated cirrhosis is similar to that in patients with decompensated cirrhosis. Among patients with compensated cirrhosis, the independent predictive factors associated with an increased risk of falls during follow-up are CD and older age.

P0162

CRITICAL FLICKER FREQUENCY IN DIAGNOSIS OF MINIMAL HEPATIC ENCEPHALOPATHY IN PATIENTS WITH CIRRHOSIS: AGE-DEPENDENT EFFECT

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Background and Aims: Minimal hepatic encephalopathy (MHE) has a great sociomedical relevance. Currently psychometric tests and critical flicker frequency (CFF) are widely used to diagnose MHE. It is well known that, there are age-based normative data of psychometric tests, but not for CFF. According to several studies there is age among of individual parameters affecting the CFF results. The aim of the study was to evaluate CFF in different age groups in controls and compare CFF in patient with HE with controls age-adjusted data.
Methods: We performed CFF analysis and psychometric tests (digit symbol test and number connection test) on 104 patients with cirrhosis (40 men, 64 women, age 24–74) and 302 without (controls). Patients in control group (137 men, 165 women, age 18–83) were divided into 6 age groups with 10 years intervals about 50 people in each. Patients with liver cirrhosis were also evaluated using West Haven criteria for overt hepatic encephalopathy (HE).
Results: CFF frequencies in the control population depended on age groups. The values peak of CFF frequency (mean±1SD) was between the ages of 18 and 30 years old (45.1±2.9 Hz), and then begin to drop: 31–40 years old 44.0±2.9 Hz, 41–50 years old 44.2±3.7 Hz, 51–60 years old 42.4±3.2 Hz, 61–70 years old 41.2±3.5 Hz; CFF values were the least in persons more than 71 year old 40.5±4.2 Hz ($p < 0.001$). There was heterogeneous CFF in people more than 50 years old: arterial hypertension, ischemic heart disease and diabetes were factors associated with decreasing CFF. Among 102 patients with cirrhosis overt HE was diagnosed in 39 patients (according West Haven criteria HE I grade in 27 patients, II grade in 12), MHE – in 16 patients (abnormal results of psychometric tests). CFF distinguished between patients with overt HE, MHE and without minimal or overt HE in the entire population cirrhotic patients: HE II stage 36.1±3.5 Hz, HE I grade 37.3±2.8 Hz, MHE 40.2±3.4 Hz, without MHE 42.8±2.1 Hz ($p < 0.005$). There was difference CFF in patients with MHE depending on age. Patients without MHE had similar CFF with age-matched controls.
Conclusions: In controls values of CFF decreased depending on age. Although in cirrhotic group there was considerable overlap between patients with overt HE, MHE and without MHE, decreasing trend CFF according severity of HE was found. Patients without MHE had similar CFF with age-matched controls. In patients with MHE there was difference CFF depending on age.

P0163

MONITORING HEART RATE VARIABILITY DURING ACUTE DECOMPENSATION PREDICTS MORTALITY AND POTENTIAL RISK OF RECURRENT HOSPITALISATION

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Background and Aims: In advanced cirrhosis, hospital re-admissions are common and mortality high from inter-current illness. The aim of this study was to use heart rate variability (HRV), previously correlated with cirrhosis severity, to determine patient outcome following admission to hospital with an acute decompensation.
Methods: Using a wireless-monitoring technology, Lifetouch®, we assessed HRV (via the parameter SDNN) remotely in 32 patients (69% male; mean age 51.8±12.3 years; 75% alcoholic cirrhosis) admitted to Royal Free Hospital with acute decompensation. Patients were followed until discharge and data collected on subsequent death, transplantation or last date of follow-up to assess re-admission rate and disease severity. HRV data was compared with clinical and biochemical indices and correlated to patients' outcome.
Results: HRV, as determined by SDNN, was significantly lower in patients who died or who required liver transplantation ($p = 0.02$). Lower SDNN was associated with the presence of ACLF ($p = 0.03$). SDNN inversely correlated with Child–Pugh, MELD-Na and CLIF-AD scores ($p \leq 0.0001$ for all; $r = -0.47$, -0.52 and -0.62, respectively). ROC curve analysis demonstrated utility of SDNN for predicting mortality (AUC 0.75; $p = 0.02$) and this was better than Child–

Pugh, MELD-Na and CLIF-AD in this cohort. Based on this analysis, an SDNN cut-off of 19.65 ms for predicting death was selected with sensitivity 90%, specificity 57% and NPV 92% (p = 0.01). Further application of this cut-off using time-to-event analyses showed patients with SDNN >19.65 ms had significantly longer transplant-free survival (p = 0.004). Amongst patients discharged from hospital, there was a trend to delayed readmission to hospital with an SDNN >19.65 ms (p = 0.07). Three patients died or were transplanted during their index admission and all had very low SDNN (<11 ms). 67% of patients with subsequent readmission (median 109 days from discharge) and 75% of patients with multiple admissions had SDNN <19.65 ms.

Conclusions: This study shows HRV to be an important prognostic criterion for mortality, transplant-free survival and potential risk for readmission following acute decompensation of cirrhosis. HRV using the Lifetouch device can be used remotely to allow monitoring of patients at home and therefore could be a novel approach providing warning signals to trigger early intervention.

P0164

CLINICAL SIGNIFICANCE OF SUBCLINICAL ASCITES IN KOREAN PATIENTS WITH HEPATITIS B VIRUS-RELATED LIVER CIRRHOSIS: A MULTICENTER, RETROSPECTIVE STUDY

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Background and Aims: Effective suppression of HBV DNA with antiviral therapy in HBV-related cirrhotic patients reduces the risk of hepatic events and mortality. However, clinical prognosis of patients with subclinical ascites (SA) compared to those without is still unclear. Therefore, we aimed to evaluate the clinical significance of SA in HBV-related cirrhotic patients treated with nucleot(s)ide analogues.

Methods: This multicenter retrospective study involving 8 hospitals enrolled patients with HBV-related cirrhosis in whom lamivudine (LMV) or entecavir (ETV) was started from January 2007 to December 2009. Patients were classified into 3 groups according to the degree of ascites: (1) No ascites (NA) group, no ascites detected by imaging study and no diuretics, (2) Subclinical ascites (SA) group, small amount of ascites detected by imaging study but no diuretics, (3) Clinical ascites (CA) group, either moderate/severe ascites or any grade of ascites with diuretics.

Results: A total of 501 patients were enrolled where 336 (67.7%), 51 (10.2%) and 114 (22.8%) patients were classified as NA, SA and CA groups, respectively while 100 (20%) and 401 (80%) patients received LMV and ETV, respectively. Serum alanine aminotransferase, creatinine, sodium level, platelet count and hepatic encephalopathy grade significantly differed between NA and SA group while gender, serum alpha-fetoprotein level and types of antiviral treatment differed between SA and CA groups (all, $P < 0.05$). The proportion of ETV-treated patients was significantly lower in CA group (81/114 patients, 71%) compared to NA group (275/336 patients, 82%) and SA group (44/51 patients, 86%) (both, $P < 0.05$). During 58±24 months of follow-up, 45 patients showed liver-related mortality. Survival rate was significantly lower in CA group compared to NA and SA group (both, $P < 0.001$) but there was no significant difference between NA and SA group ($P = 0.444$) even when subgroup analysis was done according to types of antiviral treatment. In univariate analysis, platelet count, INR, serum bilirubin, albumin, BUN, creatinine, sodium level, degree of ascites (CA), hepatic encephalopathy grade, MELD index, Child–Pugh grade and types of antiviral treatment were significantly associated with survival (all, $P < 0.05$). In multivariate analysis, only INR, serum albumin level and degree of ascites (CA) remained as independent factors (all, $P < 0.05$).

Conclusions: Subclinical ascites had no role in the prognosis of HBV-related cirrhotic patients treated with nucleot(s)ide analogues.

P0165

NON-MALIGNANT PORTAL VEIN THROMBOSIS IN PATIENTS WITH CIRRHOSIS. RESPONSE TO TREATMENT

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Background and Aims: The prevalence of nonmalignant portal vein thrombosis (PVT) ranges from 10 to 25% in patients with liver cirrhosis and it is associated with a worsening of its natural course. Optimal management of PVT in cirrhosis is not available in any consensus publication. Nevertheless, it seems that anticoagulation may constitute the initial treatment. *The aim* of our study was to evaluate the results of anticoagulation therapy in a group of cirrhotic patients with non-malignant PVT.

Methods: 27 cirrhotic patients with non-malignant PVT were studied retrospectively in our hospital between March 2009 and March 2014. Both diagnosis and follow-up of patients were performed by Doppler and contrast-enhanced ultrasound and by computed tomography.

Results: 27 patients (14 women, mean age: 59±11.8 years) were evaluated. All cases were incidental findings during routine ultrasonography. The mean MELD score was 10 (range: 6–22). 11% were on active liver transplant list. 26 patients received anticoagulation: 23 low-molecular-weight heparin and three oral anticoagulation. The median time from diagnosis to the initiation of treatment was 2 weeks. The outcome in 18/26 patients was recanalization, 15 complete (57.6%). The median time until achieving this complete response was 10 months (95% CI: 3–17). Rethrombosis occurred in five of the patients who had discontinued treatment after complete recanalization (35.7%). Patients with no response to treatment, did not show progression of thrombosis. Only two patients, one of them with 30,000 platelets, presented a bleeding complication (mild in both cases). No significant differences regarding the appearance of portal hypertension-related complications were observed. Patients with MELD score below 8 achieved recanalization in a significantly shorter time compared to the other patients (p = 0.04). Six patients died, four from complications of liver disease, but not related with anticoagulation. Thrombophilia testing was performed in 22 patients and five of them had a positive result: three with Factor V Leiden mutation, one with JAK2 gene mutation and another with hyperhomocysteinemia.

Conclusions: In cirrhotic patients with nonmalignant PVT, anticoagulation therapy led to recanalization in over half of cases, with a broad safety profile. Best outcomes seem to be achieved in a less advanced stage of liver disease. Due to the existing rethrombosis rate, long-term anticoagulation should be considered.

P0166

ANTICOAGULATION DOES NOT INCREASE PORTAL HYPERTENSION RELATED BLEEDING, BUT EXPOSES PATIENTS WITH CIRRHOSIS TO A HIGH RISK OF MINOR HEMORRHAGES. RESULTS FROM A COMPARATIVE COHORT STUDY

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Background and Aims: Anticoagulation with vitamin K antagonists (VKAs) is an effective and relatively safe therapy for patients with portal vein thrombosis (PVT). However, the haemorrhagic risk of VKAs in relation with the presence of cirrhosis, has poorly been investigated
Methods: We compared the VKAs-related bleeding risk in cirrhotic patients with *de novo* PVT (PVT-cohort, n = 62) *vs* non-cirrhotic patients with a thromboembolic event (TE-cohort, n = 160). Any bleeding during four years of follow-up or up to withdrawal of anticoagulation therapy, was recorded. The quality of anticoagulation control was measured by the time in therapeutic range (TTR) of the INR. Bleeding risk due to portal hypertension (PHT) in the PVT-cohort was compared with an independent series of cirrhotics with PHT unexposed to VKAs during follow-up (CH-cohort, n = 53). Major bleeding episodes under anticoagulation were intracranial or retroperitoneal events, fatal bleeding events, need of hospitalization or transfusion, otherwise they were considered minor bleedings. All patients with cirrhosis were under prophylaxis for PHT-related bleeding according to current guidelines.
Results: TE-cohort and PVT-cohort were comparable for age, sex. The mean of TTR was 67.7±20.9% for the former, 70.5±19.1% for the latter (p = 0.379) but treatment with VKAs was longer for the TE-cohort (31.1±16.9 vs 23.0±16.2 months, p = 0.001). Overall, 41 patients under anticoagulation experienced a bleeding episode (14 major/27 minor). The actuarial probability of major/minor bleedings was higher in PVT-cohort (23%/30%) than in the TE-cohort (6%/20%) (p < 0.001). However, the risk of upper-gastro-intestinal bleeding in PVT-cohort (15%) was the same as in the CH-cohort (13%) also adjusting for potential confounders, confirming the lack of impact of VKAs on the risk of bleeding due to PHT. Finally, the exclusion of the upper-gastrointestinal bleeding in the PVT-cohort led to a significant reduction of major bleedings accountable for VKAs, leaving a significant residual risk only for minor bleeding episodes (p < 0.05).
Conclusions: VKAs expose patients with cirrhosis and PVT to an additional risk of minor bleedings. This should be taken into account in future clinical studies to ameliorate the benefit/risk ratio of anticoagulation in this clinical setting.

P0167

ENDOSCOPIC VARICEAL LIGATION FOLLOWED BY ARGON PLASMA COAGULATION VERSUS ENDOSCOPIC VARICEAL LIGATION ALONE: A RANDOMIZED CONTROLLED TRIAL

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Background and Aims: After the first attack of acute variceal hemorrhage patients have a very high risk of recurrent variceal bleeding and death. Rebleeding rates after endoscopic variceal ligation (EVL) are high, thus current recommendation is to combine non selective beta-blockers (NSBB) to EVL but side effects and relative contraindications to NSBB are common and hinder treatment or require discontinuation in 15–20% of cirrhotic patients. Induction of fibrosis of distal esophageal mucosa using argon plasma coagulation (APC) may suppress capillary proliferation and invasion of perforating veins thus decreasing esophageal varices (EV) recurrence.
Methods: This study included 40 subjects with post viral liver cirrhosis and previous history of upper gastrointestinal bleeding. They were submitted for EVL and eradication of varices. After ensuring complete eradication of EV, patients were randomly assigned to either APC (group 1) or just observation (group 2). Both groups were followed up by endoscopy every 3 months for 24 months. Recurrence of EV was defined as development of EV grade 1 or more. Recurrence free rates and rebanding free rates were analysed using Kaplan–Meier statystical analysis.
Results: Post-treatment transient fever was significantly higher in group 1 while there were no significant differences in both groups regarding other post-treatment side effects and complications. None of subjects in both groups developed hemorrhage, perforation or stricture. During 2 year follow up 20% of subjects in group 1 experienced EV recurrence but no one needed rebanding. In group 2, 68.4% experienced EV recurrence (p = 0.002) and 63.2% underwent rebanding (P < 0.001) (Figure). No subject in group 1 experienced rebleeding during the 2 year follow up, while 10.5% of subjects in group 2 experienced rebleeding from EV (p = 0.231). No subject in both groups showed development of gastric varices. 3 subjects in group 1 and 4 subjects in group 2 showed development of new severe portal hypertensive gastropathy (p = 0.695).
Conclusions: APC after esophageal variceal eradication using EVL can decrease the risk of recurrence of EV and the need for rebanding. APC after EVL may be recommended in secondary prophylaxis against esophageal variceal bleeding especially in those who have contraindications, intolerant or non-compliant to NSBB.

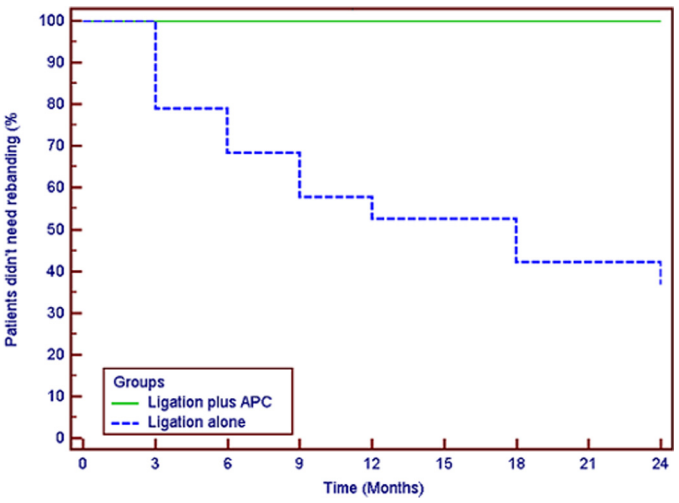

Figure: Kaplan–Meier analysis of cumulative rebanding free curves.

P0168

THE IMPACT ON HOSPITAL RESOURCE UTILISATION OF RIFAXIMIN-ALPHA FOR HEPATIC ENCEPHALOPATHY IN ROUTINE CLINICAL PRACTICE: REAL WORLD DATA FROM SEVEN UK LIVER CENTRES

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Background and Aims: Rifaximin-α (RFX) has been shown to reduce recurrence of overt hepatic encephalopathy (HE) in patients with cirrhosis. However, there is concern over the cost-effectiveness of the drug with average annual treatment with RFX costing £3,379 (€4,228). Early observational data suggested that treatment might reduce hospital admissions and decrease hospital length of stay (HLOS). The aim of this study was to determine the impact of treatment with RFX on hospital resource utilisation, using data from multiple liver centres from across the UK.
Methods: All seven participating centres agreed a standardised data collection pro-forma. Details of hospital admissions were requested at three, six and 12 months prior to RFX exposure, and for the same periods during RFX treatment. Where data were unavailable for any particular patient, data were annualised from the observation(s) provided. Clinical data were recorded at baseline, at three months, and at the end of the study period. Inpatient costs were estimated in UK£ (€@1.2×£) at 2008/9 prices from published National Health Service sources: mean £487 (€580) per day for admissions for liver disease.
Results: Data were available from 295 patients, 30% female, mean age 58 (SD 12) years, aetiologies: ARLD 60%, NAFLD 14%, viral 10%. 92% were taking concurrent lactulose. Mean baseline MELD score was 15.3 (SD 6.2). Mortality at 30 days, 90 days and 1 year were 5%, 10% and 21%, respectively, with significantly higher MELD scores at each time point (all $p < 0.001$). The mean number of hospital admissions decreased from 2.9 admissions per person per year before RFX to 1.7 during treatment (Δ 41%, $p < 0.001$). The mean hospital length of stay per admission decreased from 11.0 days before to 9.5 days during RFX treatment (Δ 13%, $p < 0.001$). This resulted in a reduction in mean annual bed occupancy from 31.7 days before to 16.4 days during RFX treatment (Δ 48%, $p < 0.001$), representing an annual reduction in inpatient liver care costs of £7,463 (€8,956) per patient. There was no change from baseline MELD at 3 months and 1 year (Δ −0.4 and −0.2 respectively). In addition, there was no clear correlation between MELD score and admissions.
Conclusions: RFX was associated with a marked reduction in the number of admissions and hospital length of stay, factors that should be taken into account when determining cost effectiveness. The likelihood of admission was independent of MELD score and the response to RFX may not be solely determined by disease severity.

P0169

CRITICAL FLICKER FREQUENCY PREDICTS SURVIVAL IN PATIENTS WITH LIVER CIRRHOSIS

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Background and Aims: Minimal hepatic encephalopathy (MHE) predicts overt hepatic encephalopathy (oHE), and this latter is associated with poor survival in patients with liver cirrhosis. However, the direct impact of MHE on the survival of cirrhotics is controversial. Therefore, we aimed to analyze the impact of MHE on long-term survival of patients suffering from liver cirrhosis.
Methods: Prospective and multicenter study including two independent cohorts of patients with liver cirrhosis. Estimation cohort (n = 151) was followed-up during 4.7±2.8 years and validation cohort (n = 117) during 4.4±3.9 years. All patients underwent: (a) critical flicker frequency (CFF) (pathological value <39 Hz); (b) Psychometric Hepatic Encephalopathy Score (PHES) (pathological value <−4 points). Outcomes to finish the follow-up were liver transplantation or death. Liver function was measured by MELD score. We used Kaplan–Meier method for univariate analysis (qualitative variables) and Cox regression for univariate (continuous variables) and for multivariate analysis.
Results: Overt HE was associated with higher mortality [42.5% (34/80) *vs.* no oHE 20.7% (38/184); logRank 26.744, $p = 0.0001$]. In the estimation cohort, univariate analysis showed that CFF <39 Hz did not predict mortality at 1 year [5.7% (2/35) *vs.* >39 Hz 1.6% (1/61); logRank 1.435, $p = 0.231$], but did at 3 years [17.1% (6/35) *vs.* >39 Hz 4.9% (3/61); logRank 4.765, $p = 0.029$] and at 5 years [28.6% (10/35) *vs.* >39 Hz 9.8% (6/61); logRank 7.835, $p = 0.005$] of follow-up (Table). Similarly, PHES <−4 did not predict mortality at 1 year [2.6% (1/38) *vs.* PHES >−4 4.1% (3/74); logRank 0.129, $p = 0.719$], but did at 5 years [34.2% (13/38) *vs.* PHES >−4 14.9% (11/74); logRank 4.944, $p = 0.026$]. In the multivariate analysis, CFF <39 Hz [HR 4.36 (95% CI 1.67–11.37); $p = 0.003$], age [HR 1.07 (95% CI 1.02–1.12); $p = 0.009$] and MELD score [HR 1.40 (95% CI 1.21–1.63); $p = 0.0001$] predicted independently the mortality. In the validation cohort, findings were very similar. In univariate analysis, CFF <39 Hz, PHES <−4, age, MELD score and a previous event of oHE were related to increased mortality. In multivariate analysis, CFF <39 Hz, age and MELD score remained independently associated with mortality.
Conclusions: Critical flicker frequency, together with age and MELD score, predicted mortality in two independent cohorts of patients with liver cirrhosis. Controversy about the impact of minimal hepatic encephalopathy on survival could be associated with a short follow-up of studies.

	Mortality		
	1 year	3 year	5 year
CFF <39 Hz	5.7% (2/35)	17.1% (6/35)	28.6% (10/35)
CFF >39 Hz	1.6% (1/61)	4.9% (3/61)	9.8% (6/61)

P0170

EFFECTS OF NUTRITIONAL THERAPY ON COGNITIVE FUNCTIONS AND HEALTH RELATED QUALITY OF LIFE IN PATIENTS OF LIVER CIRRHOSIS WITH MINIMAL HEPATIC ENCEPHALOPATHY – AN OPEN LABEL RANDOMIZED CONTROLLED TRIAL

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Background and Aims: Minimal hepatic encephalopathy (MHE) impairs health related quality of life (HRQOL), predicts development

of overt hepatic encephalopathy (HE) and associated with poor prognosis. Preliminary studies showed improvement in cognitive functions and HRQOL with nutritional supplementation in MHE but there is no study on nutritional management in patients with MHE. We assessed the effects of nutritional therapy on cognitive functions and HRQOL in patients of cirrhosis with MHE.

Methods: Consecutive patients of cirrhosis with MHE were randomized to group A received nutritional therapy (30–35 kcal/kg/day and 1.0–1.5 gram of vegetable protein/kg/day) and group B on no nutritional therapy (diet as patient was taking before) for 6 months. MHE was diagnosed based on psychometry hepatic encephalopathy score (PHES) and HRQOL assessed by Sickness impact profile (SIP) questionnaire. Primary endpoints were improvement or worsening in MHE and improvement in HRQOL.

Results: 120 patients were randomized into two groups; group-A (n = 60, age 42.1±10.3 yr, 48 men) and group-B (n = 60, age 42.4±9.6 yr, 47 men). There was no significant difference in baseline characteristics between the two groups. Baseline PHES (−8.12±1.32 *vs* −8.53±1.38; p = 0.08) and SIP score (14.25±5.8 *vs* 15.44±5.03; p = 0.85) were similar in both the groups. Reversal of MHE was higher in group A (71.1% *vs* 22.8%; p = 0.001) and improvement in HRQOL was also higher in group A (Δ SIP 3.24±3.63 *vs* 0.54±3.58; p = 0.001) compared to group B.

Conclusions: Nutritional therapy is effective in treatment of MHE and associated with improvement in HRQOL.

P0171
INVASIVE FUNGAL INFECTIONS IN PATIENTS WITH CIRRHOSIS – SUBANALYSIS OF THE FUNGAL INFECTION RISK EVALUATION (FIRE) STUDY

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Background and Aims: Infections in patients with cirrhosis are associated with increased risk of complications and mortality. Fungal infections are poorly defined in this cohort particularly the lack of distinction between colonization and invasive disease. The aim of this study was to assess the incidence and implications of invasive fungal disease (IFD) in patients with cirrhosis admitted to intensive care unit (ICU).

Methods: This is a sub-analysis of the large multi-centre Fungal Infection Risk Evaluation (FIRE) study. Clinical and laboratory parameters for 1655 patients with cirrhosis were analyzed and compared with 967 patients with very severe cardiovascular disease (CVD). The incidence of IFD and potential associations with outcomes were assessed. IFD was defined as a blood culture or sample from a normally sterile site that was positive for fungi.

Results: Variceal bleeding and pneumonia accounted for the main indication for ICU admission in liver and CVD patients respectively. CVD patients were older and had more co-morbidities (diabetes mellitus, end-stage renal disease and severe respiratory disease), and higher APACHE II score (22 vs 20; p < 0.0005). Liver patients had more invasive lines and urinary catheters, and received systemic antimicrobials more frequently.

While the incidence of IFD was similar in the two groups (0.6% in liver patients, 0.7% in CVD; p = 0.175), fungal colonization was more common in liver patients (14.2% vs 7.7%; p < 0.0005). Liver patients exhibited higher ICU and in-hospital mortality (5% vs 3.3%; p = 0.05, and 7.7% vs 4.6%; p = 0.002, respectively), and longer ICU length of stay (LOS) (70 vs 51 hours; p < 0.0005). Fungal colonization, and in particular more than one fungal isolates, was associated with higher ICU and hospital LOS but not mortality, whereas no correlation existed between IFD and clinical outcomes. Higher proportion of liver patients received antifungal therapy (15.4% vs 4.1%; p < 0.0005).

Conclusions: The prevalence of fungal colonization is high in patients with cirrhosis and is associated with higher LOS. Whether this is a marker of disease severity or has a direct effect on clinical outcomes needs further evaluation. Similarly, the role of antifungal therapy to prevent or treat fungal colonization in these patients also remains unclear. Contrary to popular belief and evidence from small studies, the incidence of IFD in patients with cirrhosis is low and not associated with mortality or LOS.

P0172
PROTON PUMP INHIBITORS INCREASE THE RISK FOR HEPATIC ENCEPHALOPATHY IN CIRRHOSIS

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Background and Aims: Proton pump inhibitors (PPIs) are commonly prescribed to cirrhosis patients. PPIs predispose to bacterial overgrowth, translocation and spontaneous bacterial peritonitis. Our aim was to determine whether PPI use is a risk factor for hepatic encephalopathy (HE).

Methods: We used the complete original data from three randomized trials of satavaptan treatment of ascites. We included all 865 patients who had not previously had HE. They were examined for HE every four weeks for 52 weeks, and satavaptan did not affect their HE risk. We used Cox regression to compare HE rates between users (340 users at entry and further 108 during follow-up) and nonusers of PPI, adjusting for gender, age, cirrhosis etiology, MELD score, variceal bleeding, P-sodium, -albumin, -platelets, and use of lactulose and diuretics. These data were updated at every visit.

Results: There were 189 HE episodes during the follow-up. The risk of developing an HE episode was 24% for those who used PPIs at inclusion vs. 20% for those who did not. Current PPI use also increased the HE rate (adjusted hazard ratio = 1.36, 95% CI 1.01–1.84), and even more so when we counted only the 92 HE episodes of grade 2 or higher (adjusted hazard ratio = 1.81, 95% CI 1.17–2.86).

Conclusions: More than half of the patients used PPIs, and they had higher HE risk after adjustment for other risk factors. These findings may serve as a warning against indiscriminate prescribing of PPIs to cirrhosis patients.

P0173
PREDICTORS OF MORTALITY IN CIRRHOTIC PATIENTS ADMITTED TO INTENSIVE CARE UNIT: COMPARISON OF DIFFERENT SCORING SYSTEMS INCLUDING THE NEW ROYAL FREE HOSPITAL SCORE

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Background and Aims: The Royal Free Hospital (RFH) score was recently developed and shown to be a better predictor for in-hospital mortality than traditional scores in cirrhotic patients admitted to the intensive care unit (ICU). However, its performance in predicting 90-day mortality has not been studied. Our aim was to evaluate the RFH score in predicting 90-day mortality and

externally validate its predictive value for in-hospital mortality. We also compared the RFH score with Acute Physiology and Chronic Health Evaluation (APACHE) III, Sequential Organ Failure Assessment (SOFA), Model for End-Stage Liver Disease (MELD) and MELD-Na scores.

Methods: We included 314 consecutive cirrhotic patients admitted to the ICU between 2003 and 2013 who had available laboratory parameters to calculate the RFH score, which comprises bilirubin, international normalized ratio, lactate, alveolar-arterial partial pressure oxygen gradient and blood urea nitrogen. Associations between the initial scores at time of ICU admission and 90-day and in-hospital mortality were calculated using logistic regression analysis. Performances of the RFH, APACHE III, SOFA, MELD and MELD-Na scores in predicting mortality were then compared using the area under the receiver operating characteristic curve (AUC).

Results: Mean age was 59±12 years and 58% were male. Etiologies of cirrhosis were alcoholic (34%), hepatitis C (29%) and non-alcoholic fatty liver disease (23%). Diagnoses during ICU admission were hepatic encephalopathy (41%), septic shock (26%), spontaneous bacterial peritonitis (12%), acute variceal bleeding (11%) and hepatorenal syndrome (10%). Of all patients, 66%, 54% and 23% required mechanical ventilation, vasopressor and renal replacement therapy, respectively. The 90-day mortality was 45% and in-hospital mortality was 29%. The AUC of RFH score in predicting 90-day and in-hospital mortality were 0.75 and 0.76, respectively. However, in comparison between scores, RFH score was not superior to APACHE III, SOFA, MELD or MELD-Na scores, ($p > 0.05$ for comparison to the RFH score), (Table).

Conclusions: The RFH score is a good predictor for both in-hospital and 90-day mortality among cirrhotic patients admitted to the ICU, but the RFH score was not superior to others. Since the arterial blood gas is not required for calculating MELD and MELD-Na scores, there may be more utility in using these scores in patients who otherwise do not require arterial blood gas measurement for other clinical indications.

Table: Comparison of receiver operating characteristic curves for RFH, APACHE III, SOFA, MELD and MELD-Na scores in predicting the 90-day and in-hospital mortality in cirrhotic patients admitted to the ICU

Prognostic model	90-day mortality		In-hospital mortality	
	AUC (95% CI)	P value	AUC (95% CI)	P value
RFH score	0.75 (0.69–0.79)	Reference	0.76 (0.70–0.80)	Reference
APACHE III score	0.73 (0.68–0.78)	0.60	0.74 (0.68–0.78)	0.52
SOFA store	0.72 (0.67–0.77)	0.46	0.79 (0.74–0.83)	0.42
MELD score	0.77 (0.71–0.81)	0.51	0.76 (0.71–0.80)	0.93
MELD-Na score	0.76 (0.71–0.81)	0.52	0.75 (0.70–0.80)	0.95

Abbreviations: RFH, Royal Free Hospital; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; MELD, Model for End-Stage Liver Disease; Na, sodium.

P0174

SOLUBLE CD163 (SCD163) IS A MARKER OF INFECTION IN PATIENTS WITH CIRRHOSIS AND ACUTE DECOMPENSATION AND AN INDEPENDENT PREDICTOR OF THE SHORT-TERM MORTALITY

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Background and Aims: sCD163 is shed from macrophages by a protease related mechanism in response to inflammatory stimuli and has benn suggested to modulate the inflammatory response. We aimed to determine the predictive potential of sCD163 levels in the determination of disease phenotype and disease course in a prospective referral cirrhotic cohort.

Methods: 378 consecutive patients with cirrhosis (LC) of different etiology (54.0% males, 70.6% alcoholic) and severity (ChildA/B/C: 39.2/38.1/22.7%, acute decompensation [AD]: 48.9%) were enrolled and followed until death or last attendance. Serum levels obtained at enrollment were assayed for sCD163 by ELISA. Detailed clinical phenotypes regarding first decompensation event (ascites formation, variceal bleeding [VB], hepatic encephalopathy or systemic bacterial infection [INF]), development of hepatocellular carcinoma [HCC] and mortality were determined prospectively during the follow-up (median [IQR], 778 [182–1720] days). Control group comprised 150 healthy subjects (HC).

Results: Serum levels of sCD163 were significantly higher in patients with LC compared to HC (median, 3724 vs. 1104 ng/ml, $p < 0.001$). In LC, sCD163 levels were associated to disease severity, as rated by the Child–Pugh stage ($p < 0.001$) but not to the presence of varices or prior VB. In non-AD patients, sCD163 levels were not able to predict the advent of the first decompensation events, development of HCC and also not the long-term mortality. In patients with AD episodes, sCD163 levels were significantly higher compared to non-AD patients but only in the presence of INF (AD-INF: 4969, AD-NON-INF: 3497 and NON-AD: 3471 ng/ml, $p < 0.001$ for both). Furthermore, during INF episodes ($n = 119$), sCD163 levels were significantly higher in those complicated with organ failure (31%) and increased gradually according to ACLF grade (No-ACLF: 4121, ACLF gr1: 7335, gr2: 7490, gr3: 12610 ng/ml, $p = 0.001$). Rate of 28-day mortality was higher among patients with sCD163 level >7110 ng/ml compared to those with ≤7110 ng/ml (46.5% vs. 15.8%, $p < 0.001$). This cut-off level of sCD163 was associated with a shorter time to death (pLogRank <0.001) in Kaplan–Meier analysis and was identified as an independent predictor in multivariate Cox regression model (HR: 2.91, 95% CI: 1.34–6.32, $p = 0.007$) comprising age, gender, etiology, co-morbidity and MELD score as covariates.

Conclusions: Admission sCD163 levels may be an additional help in rapid identification of patients with high-risk for death during AD episodes complicated with INF in LC.

P0175

ASSESSMENT OF SERUM GLYCOMICS (GLYCOCIRRHOTEST) FOR RISK PREDICTION OF HEPATOCELLULAR CARCINOMA DEVELOPMENT IN PATIENTS WITH CIRRHOSIS

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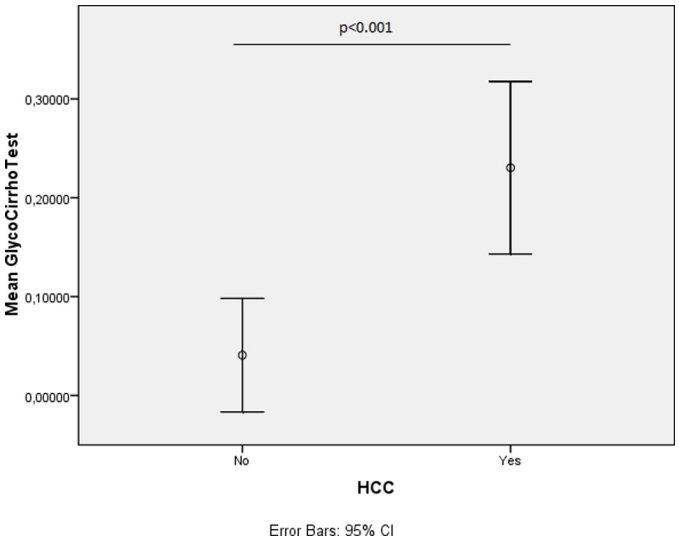
Background and Aims: Cirrhosis is a major predisposing factor for the development of hepatocellular carcinoma (HCC) with a yearly incidence ranging from 1 to 8%. EASL guidelines recommend systematic screening with liver ultrasound at 6 months interval in cirrhotic patients. GlycoCirrhoTest, based on to the respective abundance of bisecting GlcNAc residues and triantenarry glycans on serum proteins, has shown a 79% sensitivity and 86% specificity for the diagnosis of cirrhosis among patients with chronic liver diseases. The aim of the present study was to determine whether serum glycomics are predictive for the development of HCC in patients with compensated cirrhosis.

Methods: We analysed blood samples of 132 cirrhotic patients collected between 1995 and 2005. All patients had biopsy proven cirrhosis at the moment of serum sampling (Child A or B). Seventy percent of the patients had HCV infection. In the remaining patients,

the cause of cirrhosis was HBV infection, alcohol and autoimmune diseases. The patients were followed until the appearance of a HCC, death or liver transplantation. At the moment of serum sampling there was no evidence of HCC. GlycoCirrhoTest was performed using capillary electrophoreses as previously described by Callewaert et al. (Nature Medicine 2004).

Results: Thirty-five (26.5%) of the patients developed a HCC after a median follow up of 4 years (IQR: 3.6–8.06). Mean follow up in the patients who did not develop HCC was 3.7 years (IQR: 3.4–9.9; ns). There was a significant increase of the mean baseline GlycoCirrhoTest value in the patients who developed a HCC during follow up ($p < 0.001$) as compared to those who did not (Figure 1). ROC curve analysis showed an AUC of 0.716 (95% CI: 0.611–0.820) for the prediction of HCC in the patients with a follow up of at least 1 year. An 0.1 increase in the value of the GlycoCirrhoTest was associated with a 27% increase in the risk for developing HCC (OR 1.27; 95% CI: 1.098–1.475). In 81 patients (65 without HCC and 16 with HCC) baseline AFP values were available. Combining GlycoCirrhoTest and AFP using logistic regression increased the predictive value (AUC = 0.789; 95% CI: 0.677–0.900).

Conclusions: This study suggests that glycomics analysis could represent a useful biomarker for the identification of patients with cirrhosis at high risk for developing HCC. GlycoCirrhoTest may help stratify cirrhotic patients according to the risk of HCC and optimize screening.



P0176

COPEPTIN IS AN INDEPENDENT PROGNOSTIC FACTOR IN LIVER CIRRHOSIS

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Background and Aims: Copeptin is a stable cleavage product of the arginine vasopressin (AVP) precursor and is equimolarly secreted with AVP. Copeptin is currently considered as a reliable prognostic marker in a wide variety of diseases. In liver cirrhosis, however, the prognostic significance of copeptin remains unknown. We aimed to investigate the association between the severity of cirrhosis and serum copeptin concentration and to evaluate the prognostic value of serum copeptin concentration in cirrhotic patients on one-year mortality.

Methods: Cirrhotic patients hospitalized in two tertiary referral centres in the Netherlands and France were studied. Serum copeptin concentration at admission was measured using an assay in the chemiluminescence-coated tube format. The Kruskal–Wallis test was used to evaluate the effect of the severity of cirrhosis on copeptin concentrations. Cox proportional hazard regression analysis was performed to evaluate the effect of serum copeptin concentration, laboratory and clinical data on outcome. The optimal cut-off point of serum copeptin concentration was determined using the Youden Index. With the use of this cut-off point, one-year survival analysis stratified according to serum copeptin concentrations was performed using Kaplan–Meier analysis.

Results: Copeptin was measured in 186 cirrhotic patients (43 Child–Pugh A, 69 Child–Pugh B and 72 Child–Pugh C). At one year of follow-up, 35 (18.8%) patients had died and 41 (22.0%) were transplanted. Deceased or transplanted patients had significantly higher serum copeptin concentrations than the survivors [median (IQR): 17.1 (6.9–36.9) vs. 11.2 (5.2–28.9) vs. 8.2 (4.7–14.9) pmol/L, $p < 0.01$]. Median copeptin concentrations increased significantly and gradually through Child–Pugh classes A, B and C [5.4 (3.1–10.7) vs. 9.6 (6.0–17.6) vs. 13.8 (5.8–34.1) pmol/L, $p < 0.01$]. In univariate analysis, a high serum copeptin concentration (i.e., >12.3 pmol/L) showed a significant association with one-year mortality (HR = 3.35, 95% CI 1.69–6.66, $p < 0.01$, Kaplan–Meier, see figure). In multivariate analysis, patients with a high serum copeptin concentration displayed higher one-year mortality rates after adjusting for MELD (HR = 2.46, 95% CI 1.21–5.00, $p = 0.01$) or MELD-sodium score (HR = 2.80, 95% CI 1.39–5.60, $p < 0.01$).

Conclusions: Serum copeptin concentrations increase along with the severity of liver cirrhosis. Moreover, a high serum copeptin concentration was found to be of prognostic significance in hospitalized cirrhotic patients.

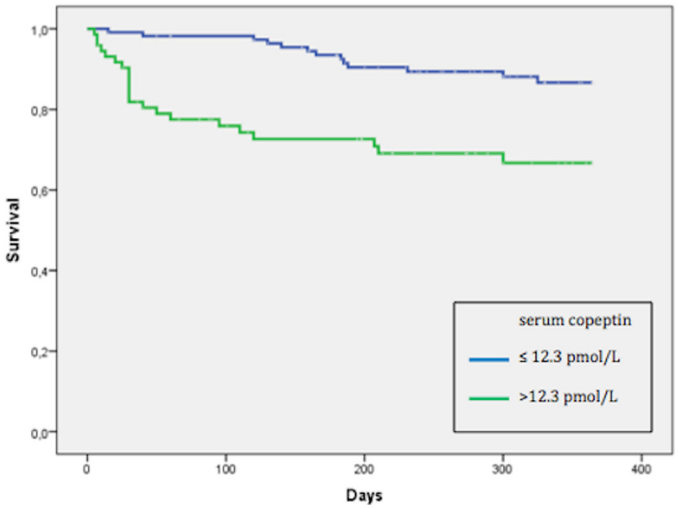

Figure: One-year survival of 186 cirrhotic patients stratified according to serum copeptin concentrations (pmol/L).

P0177

A SIMPLE OPERATIVE CRITERION TO ASSESS PATIENTS WITH LOW GRADE OF HEPATIC ENCEPHALOPATHY

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Background and Aims: The term 'covert hepatic encephalopathy' (CHE) encompasses a spectrum of conditions ranging from subclin-

ical to mild clinical alterations which do not meet the criteria for a diagnosis of overt HE (OHE). The tools to screen for CHE have not been defined. Thus, the aim of this study was to test the usefulness of the Animal Naming Test (ANT), i.e. the enumeration of the maximum number of animals in 1 min, within this clinical contest.

Methods: 208 healthy subjects stratified by decade of age (mean age 54±19 years) and 327 consecutive patients with cirrhosis (172 in Padua and 155 in Rome) underwent the ANT. Patients with cirrhosis were also assessed by the Psychometric Hepatic Encephalopathy Score (PHES), which was scored based on Italian norms and utilized to define CHE. A subgroup of 146 patients underwent a quantified electroencephalogram (EEG) and critical flicker frequency (CFF). Clinical assessment for the presence/grade of OHE was performed by expert clinicians.

Results: In controls, the ANT was found to be influenced only by extremely low levels of education (<7 yrs; $p < 0.001$) and older age (>80 yrs; $p < 0.001$). Of patients with cirrhosis, 169 were qualified as unimpaired, 126 as having CHE (abnormal PHES) and 32 as having OHE (grades II or III). Patients with CHE had significantly lower ANT performance than unimpaired patients (11±1 *vs* 15±1; $p < 0.001$). OHE patients had significantly worse ANT performance than CHE patients (3±1 *vs* 11±1; $p < 0.001$). Based on the above results, a simpler model was devised qualifying a patient as having CHE if ANT≤10 (or ≤7 for patients with less than 7 yrs of education or over 80 yrs of age; ≤4 for patients with less than 7 yrs of education and over 80 yrs of age). This adjusted threshold had 23% (95% CI=16–30) sensitivity and 91% (95% CI=87–95) specificity for CHE diagnosis. MELD score and a history of OHE were found to be independent predictors of abnormal ANT ($p < 0.01$). A significant correlation was observed between ANT performance and EEG spectral parameters (mean dominant frequency: r = 0.23 $p < 0.001$; relative theta power: r = −0.23 $p = 0.005$) but not with CFF (r = −0.009 $p = 0.93$).

Conclusions: The simple ANT test, which does not even require paper and pencil and takes 1 minute to perform and virtually no time to score, can be used as an operative criterion to improve the clinical assessment of CHE.

P0178

SARCOPENIA AND MALNUTRITION PREDICT EARLY POST-LIVER TRANSPLANTATION OUTCOMES INDEPENDENTLY OF THE MELD SCORE: IMPLICATIONS FOR ORGAN ALLOCATION

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Background and Aims: Although malnutrition and sarcopenia are prevalent in cirrhosis, their impact on clinical outcomes following liver transplantation (LT) is not well documented. We evaluated the associations of nutritional status and sarcopenia with in-hospital overall infections, time on mechanical ventilation (MV), and length of ICU and hospital stay post-LT.

Methods: We included 232 consecutive LT recipients (Males: 162, mean age: 53 years) with available pre-LT CT scan including the third lumbar (L3) vertebrae. The Royal Free Hospital-Global Assessment index (RFH-GA) was used for nutritional assessment and the L3-psoas muscle index (L3-PMI) was used for assessing sarcopenia.

Results: A wide range of RFH-GA and L3-PMI values were observed within similar MELD sub-categories (Figure 1). Malnutrition and sarcopenia were independent predictors of all evaluated outcomes. Post-LT infections were associated with MELD (OR = 1.055, 95% CI 1.002–1.11) and severe malnutrition (OR = 6.55, 95% CI 1.99–21.5) as determined by RFH-GA; MV >24 hours with MELD (OR = 1.1, 95% CI 1.036–1.168), severe malnutrition (OR = 8.5, 95% CI 1.48–48.87) and suboptimal donor liver (OR = 2.326, 95% CI 1.056–5.12); ICU stay >5 days with age (OR = 1.054, 95% CI 1.004–1.106), MELD (OR = 1.137, 95% CI 1.057–1.223) and severe malnutrition (OR = 7.46, 95% CI 1.57–35.43); hospital stay >20 days with male gender (OR = 2.107, 95% CI 1.004–4.419) and L3-PMI (OR = 0.996, 95% CI 0.994–0.999). Patients at the lowest L3-PMI who received a suboptimal graft with a cold ischemia time >12 hours had longer ICU/hospital stay and higher incidence of infections.

Conclusions: Sarcopenia and malnutrition are associated with early post-LT morbidity and their extent is not reflected into current allocation scores. Our data imply that higher quality grafts should be allocated in malnourished patients, but this needs to be further elucidated.

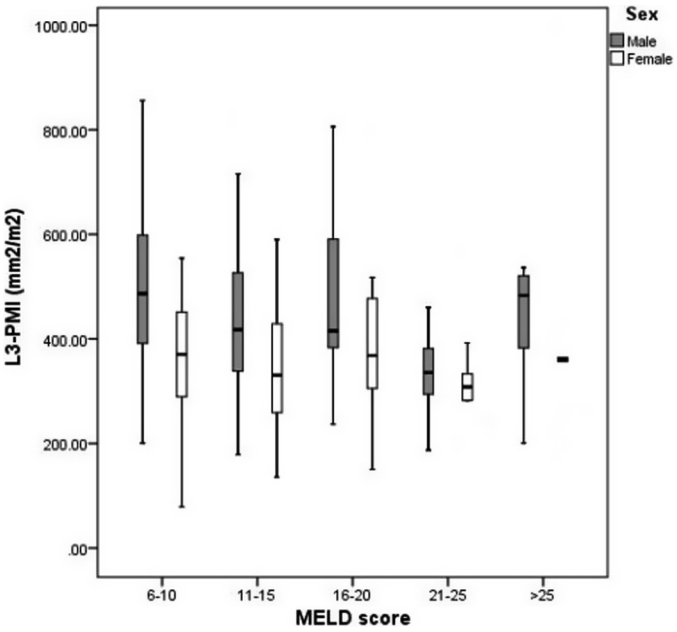

Figure 1.

P0179

THE MODEL FOR CRITICALLY ILL CIRRHOTICS (MCIC): A NOVEL PROGNOSTIC SCORE FOR PREDICTING MORTALITY IN CRITICALLY ILL CIRRHOTICS ADMITTED TO ICU

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Background and Aims: Cirrhotic patients admitted to an Intensive Care Unit (ICU) generally have a poorer outcome than patients with other illnesses. Early stratification and prediction of outcome is therefore crucial in management of cirrhosis. We prospectively studied two critically ill cirrhotic cohorts to determine prognostic variables and a prediction model.

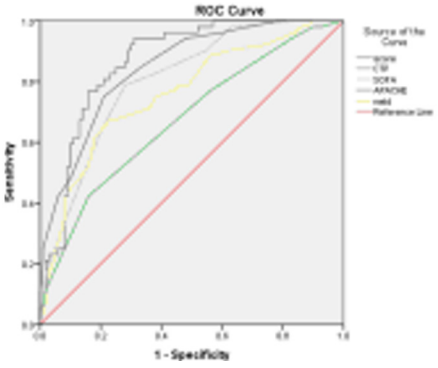
Methods: 380 consecutive cirrhotic patients admitted between (February–September 2014) to Liver ICU were followed since admission till discharge or death. The first 152 patients were evaluated and a logistic regression analysis was done to derive the predictors of mortality. A score was derived using these predictors, which was validated in a subsequent cohort of 228 patients. The predictive accuracy of this score was evaluated using AUROC.

Results: Mean age of patients was (51.2±11 and 50.4±11 yr) with male dominance (92.5% and 87.5%) in derivation and validation cohort respectively. Both cohorts were comparable in etiology of cirrhosis with majority related to ethanol (58.8%, 52%), reasons for admission to ICU, 28 daysICU mortality (34%, 37%) with sepsis

being the most common cause (78% and 81%) of death, respectively. The predictors of mortality in multivariate analysis in derivation set were baseline INR, serum creatinine (CR) and time weighted average serum lactate over first 24 hours ($\mathbf{Lac_{TW24}}$). The MCIC score was derived as an equation using forward logistic regression. The classification accuracy was 78% and 77% in derivative and validation cohorts respectively. The AUROC for the **MCIC** score was 0.87 and that for SOFA, MELD, APACHE II and CTP score were 0.80, 0.76, 0.84 and 0.67 respectively. In validation cohort, the AUROC for MCIC was 0.86, SOFA: 0.82, MELD: 0.73 and APACHE: 0.75. The dynamic blood lactate level as assessed by $\mathbf{Lac_{TW24}}$ was an important independent predictor of mortality with AUROC of 0.75 and enhanced the predictive accuracy of MELD – $\mathbf{Lac_{TW24}}$: 0.78, SOFA – $\mathbf{Lac_{TW24}}$: 0.86, and APACHE – $\mathbf{Lac_{TW24}}$: 0.83.

Conclusions: The MCIC score is the first model using dynamic lactate index in addition to liver specific INR and associated organ failure i.e. creatinine, in predicting early mortality among critically ill cirrhotic patients. This score has a better discriminative ability than the currently used indices to predict mortality and need for emergency liver transplantation, which may be useful in clinical decision-making

Validation set	Cut off	Sensitivity	Specificity	PPV	NPV
MCIC	>.55	78	76	68	84
SOFA	10.5	75	72	68	83
APACHEII	16	75	76	58	78
MELD	26	73	73	64	80
CTP	12	75	50	50	78



P0180
INCREASE IN GUT PERMEABILITY IS AN EARLY EVENT IN CIRRHOSIS AND IS NOT ASSOCIATED WITH BACTERIAL TRANSLOCATION, SYSTEMIC INFLAMMATION OR CLINICAL FEATURES: A PROSPECTIVE LONGITUDINAL STUDY

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Background and Aims: In cirrhosis, bacterial translocation (BT) is an early event and predicts occurrence of complications such as infection and mortality. In advanced liver failure, this is associated with increased gut permeability (GP) but the data in well-compensated cirrhosis is lacking. The aim of this study was to determine whether (a) GP is altered in patients with well-compensated cirrhosis (b) this was associated with bacterial translocation and (c) complications of cirrhosis.

Methods: Fifty-two stable cirrhotic patients (male: 33; age: 57.1±8.36; Child A: 84.6%, Child B: 15.4%) were included and studied longitudinally at the time of inclusion and 1-month afterwards. Clinical and biochemical variables and complications were recorded. GP was measured using standard sugar probe-based assays. Bacterial DNA was measured in serum samples by PCR reaction for the 16S ribosomal RNA gene.

Results: 19 patients (36.5%) had an abnormal GP (>0.033). There are no differences in gender, age, aetiology; diabetes, WCC, MELD (9.0±2.7 vs 9.4±3.5) and complications of cirrhosis (infection: 18.2% vs 10.5%, ascites: 54.5% vs 31.6%; HE 15.2% vs 10.5% and varices: 42.4% vs 52.6%). Only 2 patients showed evidence of BT. At 1-month, in patients with a GP < 0.033, 69% showed an increase, 7 (24.1%) a decrease and 2 were unchanged in the GP rate. In patients with abnormal GP, it worsened in 31.3%, and in 68.7% it improved. The two patients that showed evidence of BT became negative after one month and both of them displayed an improvement of their GP. 3-patients became positive after 1-month, with two showing worsening of GP. Conversely, all of these changes were not associated with any complications of cirrhosis.

Conclusions: The data of this prospective longitudinal study shows that alteration in GP occurs in a third of well-compensated cirrhosis and is a dynamic abnormalities that improves or worsens with no apparent clinical sequaela. Increased GP is not associated with evidence of bacterial translocation, clinical characteristics or systemic inflammation in well-compensated cirrhosis. It is likely that increased gut permeability is an early event but bacterial translocation in humans occurs late and factors other than translocated bacteria such as its products or metabolites contribute to clinical manifestations.

P0181
DEVELOPMENT AND VALIDATION OF A MATHEMATICAL FORMULA TO ESTIMATE GFR IN PATIENTS WITH CIRRHOSIS

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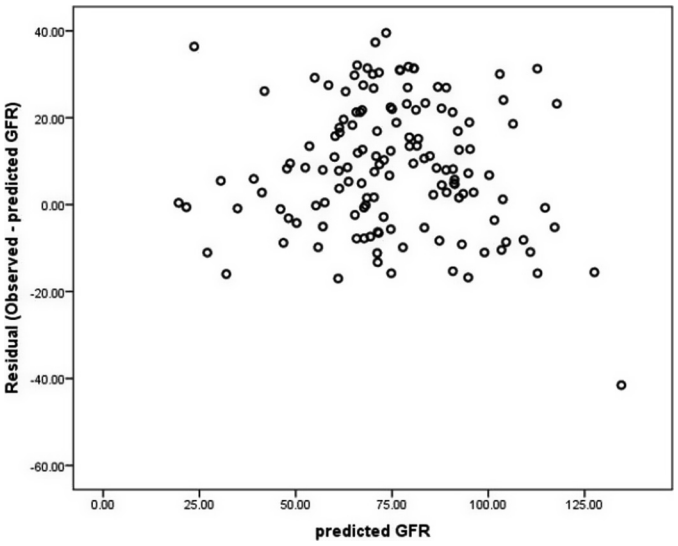
Background and Aims: Serum creatinine overestimates renal function in patients with cirrhosis leading to significant differences between "true" and calculated (MDRD equation) glomerular filtration rate (GFR). We compared the performance of MDRD with "true" GFR and the impact of this difference on MELD calculation. We further developed and validated an equation for GFR calculation in cirrhosis.

Methods: We included 469 consecutive patients (mean age: 53±11, males: 69%), who had a transplant work-up assessment between 2011–2014. "True" GFR was estimated using the gold standard of Tc-99m DTPA, which includes bolus administration of the radioactive tracer, followed by serial serum measurements at 2, 4, 8 and 24 hours according to a technique validated in patients with ascites. A corrected "true" creatinine was derived from the DTPA GFR after application of the MDRD formula. Subsequently, a corrected MELD was calculated and was compared with the conventionally calculated MELD. Stepwise multiple linear regression on log-transformed data was used to derive a GFR equation. This was compared with the observed GFRs in a validation set of 138 patients with cirrhosis assessed between 2006 and 2011.

Results: Mean DTPA GFR was 66.75±25 1.73/ml/m^2. A difference >20 ml/min/1.73 m^2 between MDRD and DTPA GFR was observed in 276 (59%) patients. Such difference was independently associated with sex (OR: 4.52, 95% CI: 2.62–7.79), black race (OR: 5.55, 95% CI: 1.52–20.33), decreased creatinine (OR: 0.94, 95% CI: 0.93–0.96) and sodium (OR: 0.92, 95% CI: 0.87–0.98) levels and ascites (OR: 2.38, 95% CI: 1.36–4.16). The corrected MELD score was ≥3 points higher in 177 (37.7%) patients. The proportion of patients with ≥3 points difference progressively increased with higher MELD scores (p < 0.001). The predicted equation derived

with the maximal R^2 (75.3%) was: GFR = 62.5·(creatinine$^{-0.839}$) × (urea$^{-0.213}$) × (INR$^{-0.186}$) × (age$^{0.123}$) × (sodium$^{0.898}$) × 1.256(if male) × 0.906(if severe ascites). The mean difference between observed/predicted GFRs (residual) in the validation set was 8.6±14.9. According to the residual plots the model was a good fit.

Conclusions: Measured creatinine overestimates renal function and leads to systematic biases in MELD calculation especially in high MELD scores. We developed and validated a new well-fitted model for renal function assessment in cirrhosis. This remains to be incorporated in prognostic scores in patients with cirrhosis.



P0182

DUODENAL MICROBIOTA IS NOT THE ORIGIN FOR BACTERIAL TRANSLOCATION IN LIVER CIRRHOSIS

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Background and Aims: Intestinal dysbiosis and bacterial translocation (BT) in cirrhosis has been regarded as the main driver for infections. Although the small intestine has been considered having the greatest potential for BT further results promoted that the colon has the largest rate of BT and permeability. We therefore investigated duodenal fluid and blood of liver disease patients regarding its bactDNA content and composition.

Methods: 156 blood and duodenal samples were collected in cirrhotics (n = 114) and controls without liver disease (n = 42). Samples were analysed for bactDNA content using quantitative 16S rRNA gene based real time PCR. To define the microbial composition of bactDNA positive samples terminal restriction fragment length polymorphism (T-RFLP) was used and clone library analyses provided the assignment of sequence types to operational taxonomic units (OTU).

Results: All 156 duodenal samples were tested positive for bactDNA with a median of 4.6×10^8 copies/ml (1.0×10^4–7.7×10^{10}). In total, 19/156 (12.2%) blood samples [cirrhosis 52.6% (n = 10) vs. non-cirrhosis 47.4% (n = 9)] were bactDNA positive (median 5.8×10^3 copies/ml; 1.7×10^3–1.5×10^5). According to T-RFLP analyses of 142/156 (91.0%) duodenal and so far 5/19 (26.3%) blood samples of patients with cirrhosis (n = 4; 80%) a division into four clusters could be performed. Cluster I (n = 10, 6.8%) revealed the lowest number of 16S rRNA gene copies (median 1.7×10^4; 3.1×10^3–7.1×10^5) and included 80% of bactDNA positive blood samples. Its microbial composition differed highly from the remaining groups. Cluster II (n = 115, 78.2%) showed two distinct OTU predominantly comprising streptococci and staphylococci and cluster III (n = 13, 8.8%) revealed a dominant OTU belonging to *Granulicatella* spp. Streptococci OTU were missing in Cluster IV (n = 9, 6.1%). Blood samples and corresponding duodenal fluids revealed in all cases (n = 5) a different microbial composition and could never be assigned to the same T-RFLP cluster.

Conclusions: The preliminary findings of different microbial clusters observed in blood and duodenal fluid from patients with cirrhosis argues against bacterial translocation originating from the duodenum.

P0183

MEASUREMENT OF SPLEEN AND LIVER STIFFNESS BY SHEAR WAVE ELASTOGRAPHY TO NONINVASIVELY EVALUATE HEPATIC VENOUS PRESSURE GRADIENT AND PORTAL HYPERTENSION

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Background and Aims: Hepatic venous pressure gradient (HVPG) is the gold standard used to determine the degree of sinusoidal portal hypertension and an important prognostic factor for patients with cirrhosis. HVPG can only be determined invasively in specialized centers. Recent data demonstrated that measurement of spleen stiffness (SS) and liver stiffness (LS) by transient elastography correlates with HVPG levels. To date, the performance of swear wave elastography (SWE) in this setting has not been reported. Therefore, the aim of the present study was to assess the diagnostic performance of SS and LS, measured by SWE, as noninvasive predictor of portal hypertension (PH) using HVPG as the gold standard.

Methods: We measured SS and LS in patients with liver disease undergoing HVPG measurement.

Results: Between September 2013 and October 2014, 63 patients were consecutively included. 67% were male and mean age was 55.3±12.4 years. Main aetiologies of liver disease were alcohol (41%), non-alcoholic fatty liver disease (29%), hepatitis B (14%) and autoimmune hepatitis (8%). Linear regression showed a significant correlation between HVPG and SS (R^2 0.433, $p < 0.001$), LS (R^2 0.468, $p < 0.0001$) and SS/LS combined (R^2 0.540, $p < 0.0001$). AUROC of SS for HVPG <10 mmHg versus ≥10 mmHg and HVPG <12 mmHg versus ≥12 mmHg was 0.86 and 0.84, respectively. A SS cut-off value of 29.6 kPa had a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 74%, 90%, 85% and 81%, and of 78%, 82%, 70% and 88% for HVPG ≥10 mmHg and HVPG ≥12 mmHg, respectively. AUROC of LS for HVPG <10 mmHg versus ≥10 mmHg and HVPG <12 mmHg versus ≥12 mmHg was 0.85 and 0.80, respectively. A LS cut-off value of 15.9 kPa had a sensitivity, specificity, PPV and NPV of 96%, 69%, 73% and 96%, and of 95%, 56%, 54% and 96% for HVPG ≥10 mmHg and HVPG ≥12 mmHg, respectively.

Conclusions: This is the first study of SWE measurement of SS and correlation with HVPG. Measurement of SS can be used for noninvasive assessment of PH, and is a slightly better predictor than LS.

P0184

HEPATOPULMONARY SYNDROME IS NOT ASSOCIATED WITH HIGHER RATES OF LEFT VENTRICLE DIASTOLIC DYSFUNCTION IN PATIENTS WITH CIRRHOSIS

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Background and Aims: Hepatopulmonary syndrome (HPS) and cirrhotic cardiomyopathy are common complications of liver cirrhosis but their real prevalence and importance are not yet known. Common pathogenetic mechanisms may be involved and some studies have shown possible links between HPS and left

ventricle diastolic dysfunction. We aimed to further investigate the relationship between HPS and echocardiographic parameters of diastolic dysfunction in cirrhotic patients.
Methods: Consecutive patients with cirrhosis and no preexisting heart or lung conditions were included in a prospective observational study. Transthoracic echocardiography (TTE) with injected agitated saline was performed in all patients. HPS was diagnosed in the presence of increased age-adjusted alveolar-arterial gradient and intrapulmonary vascular dilatations detected by contrast TTE. Diastolic dysfunction of the left ventricle was assessed through TTE measurements of left atrial volume (LAV), early (E′) and late (A′) diastolic mitral annular velocity, mitral early (E-wave) and late diastolic filling (A-wave) velocities, E/A ratio, mitral inflow E velocity to tissue Doppler E′ (E/E′) ratio, deceleration time (DT) of early filling velocity and isovolumetric relaxation time (IVRT). N-terminal prohormone of brain natriuretic peptide (NT-proBNP) levels were also measured in all patients. These variables were compared in univariate and multivariate analysis (using binary logistic regression) between patients with and those without HPS.
Results: Fifty-four patients with cirrhosis (21 female, mean age 59 years) were included. 32 were Child–Pugh class A, 12 were Child Pugh B and 10 Child Pugh C. 28 patients had intrapulmonary vascular dilatations but only 17 (31%) fulfilled all criteria for HPS. Mean NT-proBNP levels did not differ between patients with and those without HPS (260 vs. 251 pg/mL, p = 0.88 student t test). None of the evaluated echocardiographic parameters (LAV, E′, A′, E/E′, IVRT, E/A and DT) correlated significantly with the presence of HPS in either univariate or multivariate analysis.
Conclusions: In this cohort of 54 consecutive cirrhotic patients the presence of hepatopulmonary syndrome did not associate with increased rates of left ventricle diastolic dysfunction as evaluated by echocardiographic parameters or NT-proBNP levels.
Acknowledgments: This study is part of the ACCEPT initiative and POSDRU 159/1.5/S/141531.

P0185
ACUTE-ON-CHRONIC LIVER FAILURE NOT ASSOCIATED WITH BACTERIAL INFECTIONS. CHARACTERISTICS, PROGNOSIS AND IMPORTANCE OF SYSTEMIC INFLAMMATORY RESPONSE

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Background and Aims: Acute-on-Chronic Liver Failure (ACLF) is a frequent syndrome that occurs in patients with cirrhosis that is associated with high short-term mortality. Frequently, it occurs in the context of systemic inflammatory response caused by bacterial infections. However, in some cases, ACLF occurs without an identifiable bacterial infection. Although there is information about ACLF associated with infections, information about characteristics and outcome of ACLF not associated with infections is very limited. The aim of this study is to investigate the characteristics, evolution and survival of patients with ACLF not associated with infections.
Methods: We analyzed 302 patients with ACLF included in the European multicentric CANONIC study of EASL-CLIF consortium. Patients were classified into two groups according to the absence or presence of bacterial infections (ACLF-noinf and ACLF-inf, respectively). Diagnosis of bacterial infection was based on standard procedures.
Results: One-hundred and forty-three patients (47%) had ACLF-noinf, while 159 had ACLF-inf (53%). Among patients with ACLF-noinf no precipitating event could be found in 54% despite extensive assessment. In the remaining patients, precipitating events were active alcoholism, gastrointestinal bleeding or miscellaneous causes. Comparison of frequencies of organ failures included in the definition of ACLF showed a similar frequency of kidney, liver, and coagulation failures and a significantly lower frequency of brain, circulatory, and lung failures in patients with ACLF-noinf compared to patients with ACLF-inf. Patients with ACLF-noinf had higher leukocyte count and C-reactive protein levels than those of patients without ACLF, yet lower compared to values of patients with ACLF-inf. CLIF-Organ failure and CLIF-C-ACLF scores, two classifications to assess severity of ACLF, were significantly higher in patients with ACLF-inf than in those with ACLF-noinf. However, 3-month mortality of patients with ACLF-noinf was similar to that of patients with ACLF-inf, and close to 50%. In patients with ACLF-noinf leukocyte count was an independent predictive factor of 3-month mortality.
Conclusions: ACLF-noinf is common and associated with poor prognosis. Current findings suggest that ACLF-noinf occurs in the setting of a systemic inflammatory response, the severity of which is associated with prognosis. Further studies are needed to investigate characteristics of this inflammatory response and identify new therapeutic targets.

P0186
A MODEL TO PREDICT MORTALITY IN CIRRHOTICS PRESENTING WITH CELLULITIS

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Background and Aims: Infections are arguably the commonest cause of mortality in cirrhosis patients. Due to significant hemodynamic alterations, pedal edema, ascites, coagulopathy and cirrhosis associated immune deficiency syndrome, skin and soft tissue infections (SSTI) are not uncommon in cirrhotics. Prevalence and outcome of cirrhotics presenting with SSTI and predictors of mortality have not been adequately studied.
Methods: From Aug 2012 to Oct 2014, 2368 patients of cirrhosis and acute on chronic liver failure (defined as per Asian Pacific Association for Study of Liver criteria) were admitted. The electronic records were analysed retrospectively for presentation with regards to SSTI and their influence on mortality. A model was developed to predict mortality due to SSTI.
Results: Two hundred and fifty patients (9.47%, M-212, F-38) were diagnosed to have cellulitis or severe skin infections. The commonest aetiology was HBV related cirrhosis, followed by non-alcoholic fatty liver and alcoholic liver disease. Fifty-three patients died due to refractory septic shock (M-49, F-4; 21.2%). The commonest organism cultured from wound site was E. coli, followed by Klebsiella and Acinetobacter species. Commonest systemic infection associated with SSTI was pneumonia. The most

significant extracutaneous infection that predicted mortality was spontaneous peritonitis ($p < 0.001$) as was the need for surgical management ($p < 0.05$). After univariate and multivariate analysis, the significant independent predictors of mortality were presence of spur cells, persistence of toxic granulations on peripheral smear on day 3, necrotizing fasciitis at admission, mean arterial pressure (MAP) ≤62 mmHg, blood urea (BU) ≥54 mg/dL, Child–Pugh score (CTP) ≥12 and serum lactate (Slac) on day of admission >5.75 mmol/dL with each at p-value <0.05. These independently predicted mortality accurately in 97.6% of patients. Comparative ROCs of independent risk factors revealed that lactate level at admission was the predominant factor predicting mortality in cirrhosis patients with cellulitis.

Table 1. Multivariate analysis – regression model significant variables

Variables in equation								
	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for exp(B)	
							Lower	Upper
Spurcells	3.572	1.325	7.266	1	0.007	35.588	2.651	477.803
Necrosis	3.143	1.437	4.781	1	0.029	23.163	1.385	387.398
ToxicGran	2.571	1.299	3.920	1	0.048	13.083	1.026	166.769
MAP	3.389	1.116	9.221	1	0.002	29.623	3.325	263.929
BU	3.218	1.144	7.910	1	0.005	24.984	2.652	235.328
CTP	2.288	0.920	6.181	1	0.013	9.858	1.623	59.872
Lac	4.854	1.124	18.659	1	0.000	128.197	14.172	1159.609
Constant	−11.963	2.552	21.981	1	0.000	0.000		

Table 2. Area Under the Curve

Test Result Variable(s)	Area	Std error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Spurcells	0.638	0.047	0.002	0.545	0.731
Necrosis	0.713	0.046	0.000	0.623	0.803
ToxicGran	0.731	0.045	0.000	0.643	0.819
MAP	0.716	0.040	0.000	0.637	0.794
CTP	0.788	0.035	0.000	0.720	0.857
BU	0.635	0.043	0.002	0.551	0.720
Lac	0.870	0.033	0.000	0.805	0.935

Table 3. Prediction model

Observed outcome	Predicted outcome		
	0	1	Percentage correct
Alive	195	2	99.0
Dead	4	49	92.5
	Overall percentage		97.6

Conclusions: SSTI are not uncommon and if severe, carry significant mortality in cirrhotics. The regression model showed presence of spur cells, persistence of toxic granulations in the initial 72 hours after admission, low MAP (≤62), higher BU (≥52), CTP score (≥12) and high Slac at presentation (≥5.75) carry high mortality. Patients presenting with these factors at admission need to be aggressively managed with the sepsis bundles of care for early and timely salvage.

P0187

A RANDOMIZED CONTROLLED TRIAL COMPARING THE ALFAPUMP© WITH PARACENTESIS IN PATIENTS WITH REFRACTORY ASCITES: CLINICAL AND PATHOPHYSIOLOGICAL EFFECTS ON CARDIAC, HAEMODYNAMIC, INFLAMMATORY, RENAL AND NUTRITIONAL PARAMETERS

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Background and Aims: Cirrhotic patients with refractory ascites (RA) who require regular paracentesis find it distressing, is associated with poor quality of life and a high mortality. A novel, implantable, programmable device (alfapump©, Sequana Medical) that transfers ascites into the bladder to enable passing ascitic fluid into the urine has been shown to be safe in treating RA in uncontrolled studies. However, its effects on pathophysiological variables are lacking. The aims of this study were to determine the effect of the alfapump© on cardiac, haemodynamic, inflammatory and nutritional parameters.

Methods: 17 patients with RA (age: 62±7 years, 75% males) not suitable for TIPS were randomised to either the alfapump© (**AP**) or paracentesis (**P**) in a sub-study of a European trial (primary end point: paracentesis free survival). Patients were followed up for at 1 and 3-months. Cardiac hemodynamics (including cardiac output), neurohormones (BNP, renin), inflammatory markers (cytokines), urinary biomarkers (NGAL, KIM-1) and nutritional parameters were measured. Data were analysed using time dependent non-parametric statistics.

Results: 15 (**AP**: 7; **P**: 8, *2 withdrawals*) were analysed. Patients were well-matched for age, sex, aetiology and severity [MELD: **AP**: 14.5 (13–22); **P**: 15 (11–22)]. 1 patient required large volume paracentesis (LVP) in the **AP** group compared with all in the **P** group ($p < 0.001$); [LVP/month: [0 (0–0.3) in **AP**] and [1.3 (0.2–2.1) in **P**]. Transient acute kidney injury was observed in the post-operative period in all 7 **AP** patients, which resolved spontaneously, with hepatorenal syndrome in 1 **AP** patient. Serum creatinine ($p = 0.08$) and renin increased insignificantly (0.06) and albumin decreased insignificantly (0.08) in the AP group with no significant differences in cardiovascular parameters, urinary injury markers and cytokines. There was a significantly greater improvement in the estimated dry body weight ($p < 0.001$), mid-arm muscle circumference ($p < 0.001$), triceps skin fold thickness ($p < 0.001$) and hand-grip ($p < 0.001$) in the **AP** group compared with **P**.

Conclusions: The results of this study suggest that the alfapump© is safe and effective in RA and improves the nutritional state of patients with RA. Transient AKI episodes are reversible with volume expansion and the non-significant changes in creatinine and renin in AP group are related to relative reduction in serum albumin. Future studies should evaluate albumin supplementation in AP patients.

P0188

HEPATOPULMONARY SYNDROME. PREVALENCE, ASSOCIATED FACTORS AND IMPACT IN SURVIVAL AFTER LIVER TRANSPLANTATION

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Background and Aims: Hepatopulmonary syndrome is frequent in liver cirrhosis (LC) but the association between liver dysfunction and impact in survival after liver transplantation remains controversial. The aim of this study was to determine the prevalence of HPS in a cohort of patients with LC and the possible associated factors and the impact on survival at month and at year after LT.
Methods: We prospectively studied 204 patients who were evaluated for LT between November 2010 and June 2014. An arterial blood gas analysis, a transthoracic contrast echocardiography and lung functional test were performed in all patients for LT evaluation. HPS was defined by a positive contrast echocardiography and alveolar-arterial oxygen gradient ≥15 mmHg or partial pressure of arterial oxygen <80 mmHg. Demographic, clinical variables and survival at one month and one year after LT were compared in patients with and without HPS.
Results: HPS prevalence was 34.3% (44, 21 and 5 patients with mild, moderate and severe degree respectively). Women (OR = 2.4, 95% CI: 1.21–4.90), hepatocellular carcinoma (OR = 0.38, 95% CI: 0.21–0.7) and liver function measured by MELD score (16.87±5.10 vs. 13.72±5.81; p = 0.000), Child–Pugh score (8.74±2.24 vs. 7.64±2.29; p = 0.001) and their components (including ascites and encephalopathy), were associated with HPS. Only women, Child–Pugh score and INR were independently associated with HPS in the multivariate analysis. Until now, 111 patients were transplanted (38 with HPS). No differences in survival after one month and one year after LT were observed in patients with and without HPS or depending on severity of HPS.
Conclusions: Prevalence of HPS is high and is independently associated with female sex, poor liver function as reflected by Child–Pugh score and with INR. The survival among the first year after LT was not impacted by the presence of HPS or their severity.

P0189

PROTON PUMP INHIBITOR TREATMENT IS ASSOCIATED WITH INCREASED MORTALITY IN PATIENTS WITH CIRRHOSIS

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Background and Aims: In recent years the wide use of proton pump inhibitors (PPI) in patients with cirrhosis was questioned after several studies indicated an increased number of infectious complications under PPI treatment. Since the prognostic relevance of PPI treatment in patients with cirrhosis is still unknown we performed a prospective study investigating the relation of PPI treatment and overall survival (OS) in cirrhotic individuals.
Methods: Patients with cirrhosis were prospectively enrolled into the study and followed until death, liver transplantation or last contact. Overall survival was the primary end point. At the day of the study inclusion data on PPI treatment as well as further clinical and laboratory data were assessed. Univariate and multivariate Cox regression hazard models were used to identify independent predictors of survival.
Results: A total of 272 cirrhotic patients were included in the study. 213 (78.3%) patients received PPI. PPI treatment was associated with higher MELD scores (median MELD 16 vs. 12, p < 0.027) and ascites (77.0% vs. 55.9%, p = 0.039) at baseline in a multivariate logistic regression analysis. In the multivariate Cox regression model PPI use was identified as an independent predictor of mortality (hazard ratio 2.330, 95% confidence interval 1.264–4.296, p = 0.007) together with the model of end stage liver disease (MELD) score (1.097, 1.059–1.137, p < 0.001), hepatocellular carcinoma (4.971, 2.909–8.498, p = 0.001) and hepatic decompensation (2.211, 1.208–4.4045, p = 0.01).
Conclusions: Treatment with PPI was identified as an independent risk factor for mortality in patients with cirrhosis. Although the study design cannot ultimately prove a causative role for the increased mortality, the common prescription of PPI in patients with cirrhosis should be considered carefully taking into account its potential adverse effects.

P0190

PREDICTIVE MODEL OF MORTALITY IN CIRRHOTIC PATIENTS WITH HIGH RISK SPONTANEOUS BACTERIAL PERITONITIS

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Background and Aims: Hospital mortality in high-risk spontaneous bacterial peritonitis (SBP) patients is high despite albumin treatment. The aim of this study was to develop and validate a model to identify SBP patients with high risk of mortality in spite of receiving the standard treatment with antibiotics and albumin.
Methods: We analyzed all cirrhotic patients with SBP and high risk of mortality treated with albumin over a 10-year period in a third level hospital. We defined high risk of mortality when patients presented urea ≥11 mmol/L and/or bilirubin ≥68 μmol/L at SBP diagnosis. We developed a predictive model of in-hospital mortality that afterwards was validated in an external cohort from two different third level hospitals in the same area.
Results: We included 118 high-risk SBP episodes treated with albumin and antibiotics. In-hospital mortality was 33/118 (28%). The independent predictive factors of in-hospital mortality were serum urea (OR 1.131, 95% CI 1.051–1.218, p = 0.001), blood leukocyte count (OR 1.063, 95% CI 1.010–1.118, p = 0.02), Child–Pugh score (OR 1.555, 95% CI 1.114–2.171, p = 0.009) and mean arterial pressure (OR 0.947, 95CI% 0.907–0.989, p = 0.01) at SBP diagnosis. A predictive model including these four variables was established, showing a discrimination accuracy (AUC) of 0.850, 95% CI 0.777–0.922. A cut-off point of 0.245 was selected with a sensitivity of 0.85 and specificity of 0.75. In-hospital mortality was higher among patients with a model value ≥0.245 (28/49, 57.1%) than in patients with model value <0.245 (5/69, 7.2%) (p < 0.001). The validation series included 123 patients. In-hospital mortality was 33/123 (26.8%). The discrimination accuracy of the model in the validation series was 0.760, 95% CI 0.654–0.867. The in-hospital mortality was 24/57 (42.1%) and 9/66 (13.6%) in patients with a model value ≥ and <0.245, respectively.
Conclusions: A predictive model of mortality has been established and validated in SBP patients with high risk of mortality, including urea, blood leukocyte count, Child–Pugh score and mean arterial pressure. These findings may contribute to identify patients that could benefit from additional therapeutic strategies.

P0191

IFN-FREE REGIMENS OVERCOME THE NEGATIVE EFFECT OF PORTAL HYPERTENSION ON VIROLOGIC RESPONSE AND VIRAL KINETICS IN PATIENTS WITH CIRRHOSIS

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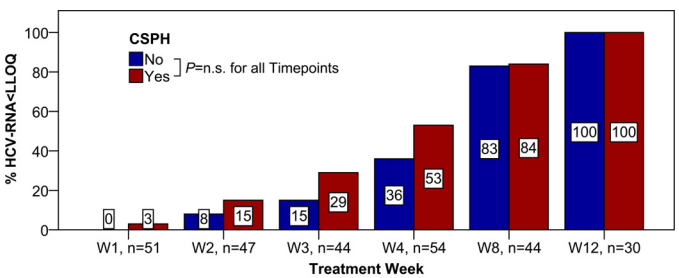
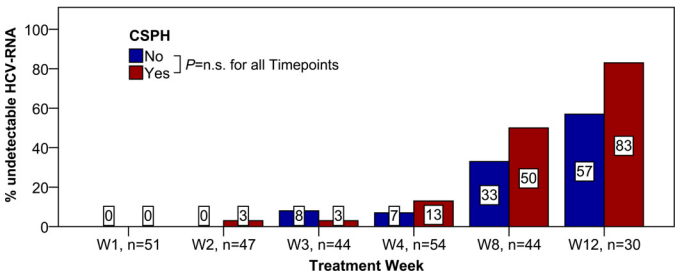
Background and Aims: Clinically significant portal hypertension (CSPH) is the strongest predictor of virologic response to pegylated interferon/ribavirin in patients with cirrhosis. However, the availability of interferon (IFN)-free regimens has ushered in a new era in the treatment of chronic hepatitis C (CHC). We aimed to investigate the impact of portal pressure assessed by hepatic venous pressure gradient (HVPG) measurement on on-treatment response and viral kinetics in patients treated with IFN-free regimens outside of clinical trials.
Methods: Fifty-six patients with CHC and cirrhosis who underwent HVPG measurement before starting an IFN-free therapy were retrospectively studied. CSPH and pronounced portal hypertension (PPH) were defined as HVPG ≥10 mmHg and ≥16 mmHg, respectively. HCV-RNA was assessed using the Abbott RealTime HCV assay with a lower limit of quantification (LLOQ) and detection of 12 IU/mL. Continuous variables were reported as mean±standard deviation or median (interquartile range).
Results: Sofosbuvir/daclatasvir, sofosbuvir/ribavirin and sofosbuvir/simeprevir were used in 32 (57%), 12 (21%) and 11 (20%) patients, respectively. One patient (2%) was treated with simeprevir/daclatasvir. CSPH and PPH were observed in 41 (73%) and 31 (55%) patients, respectively. The distribution of treatment regimens was comparable between patients with or without CSPH/PPH. Patients with CSPH/PPH had lower platelet counts and albumin levels, while bilirubin levels, INR, MELD and Child–Pugh scores were higher than in patients without CSPH/PPH. Overall, on-treatment response was impaired. At treatment week 4, only 26/54 (48%) and 6/54 (11%) patients had HCV-RNA <LLOQ and undetectable HCV-RNA, respectively. Moreover, at treatment week 12, undetectable HCV-RNA was achieved in 23/30 (77%) patients, while all patients had HCV-RNA <LLOQ. Importantly, on-treatment response and viral kinetics were neither affected by CSPH, nor by PPH at any time point.
Sustained virologic response (SVR) rates will be available at the time of the meeting.





Conclusions: This is the first study investigating the impact of HVPG on on-treatment response and viral kinetics in a real-life cohort including patients treated with a combination of two DAAs. IFN-free regimens overcome the negative effect of portal hypertension on on-treatment response and viral kinetics. The upcoming data will allow for the assessment of the impact of portal hypertension on relapse rates.

P0192

ASSESSMENT OF LIVER FUNCTION BY THE EXHALED BREATH-PRINT DURING CHRONIC LIVER DISEASE

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Background and Aims: Since the liver plays a key metabolic role, the pattern of volatile organic compounds (VOCs) in the exhaled breath might change with type and severity of liver disease. Gas sensor array technology (commonly dubbed electronic nose, e-nose) is a novel technique, which provides a sort of fingerprint of exhaled breath (breath-print, BP) by detecting VOCs through multiple sensors. It has so far never been tested in patients with chronic liver disease. We aimed to assess discriminative and classificatory properties of the e-nose in liver diseases by analysing the BP of patients affected by chronic hepatitis (CH) and by liver cirrhosis (LC) with different Child–Pugh classes (CPC).
Methods: Thirty-nine patients with CH (19 infective, 2 alcoholic and 18 metabolic) and 65 patients with LC (24 infective, 30 alcoholic and 11 metabolic), were recruited. Clinical and biochemical data were collected. Patients underwent breath collection by Pneumopipe®. Breath analysis was obtained through e-nose (gas sensor array BIONOTE), which gives a BP made up of a sequence of four 7-dimensional data arrays, composed of the responses of a 7-dimensional gas sensor array at four temperatures (50–100–150–200°C).
Results: Partial Least Square Discriminant Analysis (PLS-DA) was performed to build models able to distinguish CH from LC patients and, among LC patients, different CPC. A discriminative model for CH vs LC was obtained only after excluding patients with hepatic encephalopathy (17 subjects). The accuracy of these models were then tested for their classificatory properties, showing a sensibility, specificity and positive predictive value for LC vs CH of 74.5%, 63.6% and 66.7%, and for CPC A-B vs C of 87.5%, 64.7% and 87.5%, respectively. The 16 cirrhotic patients misclassified as CH by our model showed a trend towards a better CPC ($p = 0.07$), and a significantly higher glomerular filtration rate ($p = 0.01$), compared to correctly classified cirrhotics.
Conclusions: For the first time we report original results concerning the performance of the e-nose technology during chronic liver disease. These data are proof of concept that the e-nose could be a valid non-invasive instrument to characterize and monitor hepatic function. The observed classificatory properties might be further improved by refining stage-specific BP and considering the impact of comorbidities, such as renal failure, in a larger series of patients.

P0193

A NOVEL SCORING SYSTEM FOR PREDICTION OF CONTRAST INDUCED NEPHROPATHY IN CIRRHOSIS – THE 'CINIC SCORE'

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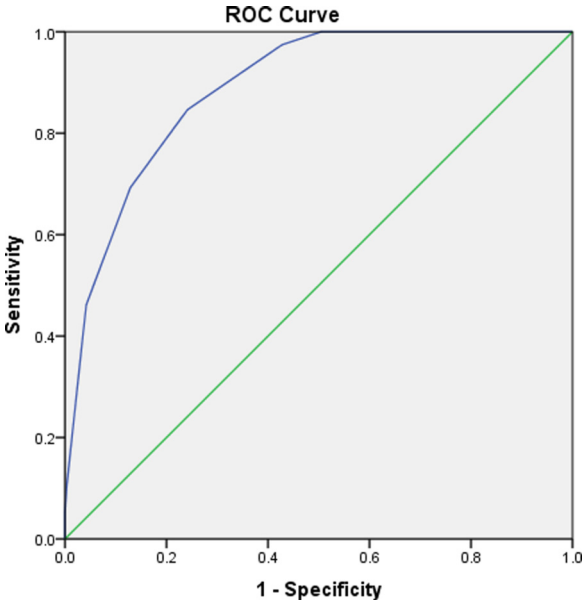
Background and Aims: Contrast induced nephropathy (CIN) is described in patients undergoing interventional cardiac procedures and a grading system for the same has been in use. The predictors of development of CIN in cirrhotics (CP) has not been studied adequately. It is well known that CP have low creatinine (Cr) clearance and renal reserve and maybe at risk, to develop CIN. We devised a novel scoring system (**C**ontrast **I**nduced **N**ephropathy **I**n **C**irrhosis, **CINIC** score) to predict CIN in CP using a derivative group (DG) which was validated in a separate cohort (VG).

Methods: DG of 350 CP [M = 281, F = 69; alcohol = 86, HBV = 190, others = 74] with or without decompensation undergoing contrast enhanced computed tomography [CECT, Omnipaque 300 (Iohexol, 1 ml/kg; 300 mg/ml Iodine)], of abdomen were prospectively studied for development of CIN. CIN was defined as an absolute increase of 0.5 mg/dL Cr from baseline or increase in Cr >25% within 48 to 72 hrs. Independent risk factors were identified on multivariate analysis, a score derived, based on regression coefficient values rounded for each variable, providing a total score. This was validated in independent VG of 150 CP undergoing CECT.

Results: 39 (11.1%) patients developed CIN. On univariate analysis severity of ascites ($p < 0.001$), hepatic encephalopathy ($p < 0.001$), diabetes (DM, $p < 0.05$), hypertension (HTN, $p < 0.01$), hyponatremia ($p < 0.01$), hypoalbuminemia ($p < 0.001$), baseline uric acid (UA, <0.001), CTP and MELD (<0.001) were significantly associated with CIN. On multivariate analysis, only UA [OR 3.089 (95% CI 1.371–6.957), $p < 0.05$], CTP [43.12 (9.29–200.19), <0.001], DM [6.828 (2.411–19.336), <0.001] and HTN [6.072 (2.650–19.336), <0.001] were found significant. Regression coefficient values for each variable were rounded off to give points – UA 1 (>6.2 mg/dL, Sen 60%), DM & HTN, 2 each, and CTP 4 (>11, Sen 93%), for a total score of 9. Prediction of CIN was made at a total score of ≥4.5, rounded off to 5 [sensitivity 84%, specificity 76%, AUROC 0.889 (95% CI 0.845–0.933), $p < 0.001$]. Patients in DG and VG were comparable. In VG, CINIC score predicted CIN with AUROC 96.3 (Sen 80%, Spe 90.4%, PPV 48%, NPV 97.6%).



Coordinates of the ROC curve

Test Result Variable(s): score Positive if Greater Than or Equal To[a]	Sensitivity	1 – Specificity
−1.0000	1.000	1.000
.5000	1.000	.797
1.5000	1.000	.627
2.5000	1.000	.505
3.5000	.974	.428
4.5000	**.846**	**.241**
5.5000	.692	.129
6.5000	.462	.042
7.5000	.103	.003
8.5000	.051	.000
10.0000	.000	.000

Area Under the Curve

Test Result Variable(s): score

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower bound	Upper bound
.889	.023	.000	.845	.933

The CINIC Score

Parameter	Score	
	Present	Absent
Baseline uric acid >6.2 mg/dL	1	0
Presence of diabetes mellitus	2	0
Presence of hypertension	2	0
Child score >11 points	4	0
Total	9	–

Score >5 predicts CIN development.

Conclusions: In CP, independent risk factors of CIN include CTP >11, DM, HTN and baseline UA (>6.2 mg/dL). The CINIC score ≥5 predicted CIN in 89% of CP, which on validation yielded an AUROC 96.3%. The presence of CTP >11 along with any other significant independent variable, or presence of all 3 independent variables apart from CTP, predicted CIN. Based on CINIC, preventive measures prior to CECT warrant evaluation.

P0194

SEVERE 25-HYDROXYVITAMIN D DEFICIENCY IS ASSOCIATED WITH INFECTIONS AND MORTALITY IN CIRRHOSIS

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Background and Aims: Vitamin D, best known to regulate bone mineralization, has numerous additional roles including regulation inflammatory pathways. Recently, an increased incidence of 25-hydroxyvitamin D3 [25(OH)D3] deficiency has been found in subjects suffering from liver diseases. We here investigated if low vitamin D levels might be associated with prognosis, inflammation and infectious complications in patients with cirrhosis.

Methods: We performed a prospective cohort study investigating the relation between 25(OH)D3 levels and stages of cirrhosis, mortality and complications of cirrhosis, including infections. 25(OH)D3 levels were quantified by radioimmunoassay from serum samples obtained at study inclusion.

Results: 270 patients with cirrhosis were enrolled into the present prospective cohort study. The mean follow-up time was 422±399 days with a range of 1–1382 days. 37 (13.7%) patients underwent liver transplantation and 85 (31.5%) individuals died within the study. The mean serum 25(OH)D3 concentration was

9.3±7.5 ng/ml with a range of 0.9 to 46.1 ng/ml. 25(OH)D3 levels differed significantly between Child Pugh scores and showed a negative correlation with the model of end stage liver disease (MELD) score. Patients with decompensated liver disease had significantly lowered 25(OH)D3 levels. 25(OH)D3 levels significantly differed between patients with infectious complications compared to those with none and individuals with SBP (spontaneous bacterial peritonitis) had significantly lower 25(OH)D3 levels compared to patients without SBP. 25(OH)D3 levels ≤6 ng/ml as well as serum 25(OH)D3 concentrations ≤10 ng/ml levels were associated with higher mortality compared to higher 25(OH)D3 levels (HR 1.82 for 6 ng/ml and HR 1.71 for 10 ng/ml, respectively).
In a multivariate Cox regression model 25(OH)D3 levels were independently associated with shorter OS.
Conclusions: 25(OH)D3 deficiency is associated with advanced liver disease and low 25(OH)D3 levels are an indicator for a poor outcome and are associated with infectious complications.

P0195
DEVELOPMENT OF A SMARTPHONE APPLICATION TO ENABLE REMOTE MONITORING IN THE OUTPATIENT MANAGEMENT OF CIRRHOTIC ASCITES

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Background and Aims: Patients who develop hepatic decompensation with ascites have a poor prognosis and often experience other complications including spontaneous bacterial peritonitis, hepatic encephalopathy and variceal bleeding. We hypothesised that smartphone (SP)-enabled remote monitoring of patients with ascites may enable early detection of infection and acute decompensation, facilitate timely intervention and improve patient outcomes. This pilot study aimed to design, develop and implement a remote monitoring system (RMS) for outpatients with cirrhotic ascites.
Methods: Surveys were undertaken with patients and hepatologists to quantify the demand for a RMS and identify issues regarding implementation. Two applications (apps) were developed – a native app for Android SP and a web-based app for all internet-enabled devices (computers, tablets, other SP). Patients used the RMS in a 6-week prospective non-randomised trial.
Results: 27 patients (mean age 56 years, 67% male, 54% Child–Pugh B, 74% alcoholic liver disease) and 5 hepatologists were surveyed. 65% of patients reported that they would use a RMS. The system schematic (Figure 1) was designed based on survey data, and an Android and a Web app were developed. 9 patients used the RMS for a mean 40.3days and entered 15±14.5 updates. A total of 13 automated alerts occurred, 85% during office hours. 30% of automated alerts resulted in clinically significant changes to management (inpatient admission n = 1, outpatient appointment n = 2, avoided admission n = 1). Isolated weight gain (3 kg) had the least predictive value for requiring intervention (12.5%); presence of symptoms had the highest predictive value for changing management (60%).
Conclusions: We have successfully designed an internet-enabled RMS for outpatients with cirrhotic ascites that could be an adjunct to nurse-led clinics. Future studies will optimise the alert thresholds, assess long-term patient adoption and quantify clinical impact.

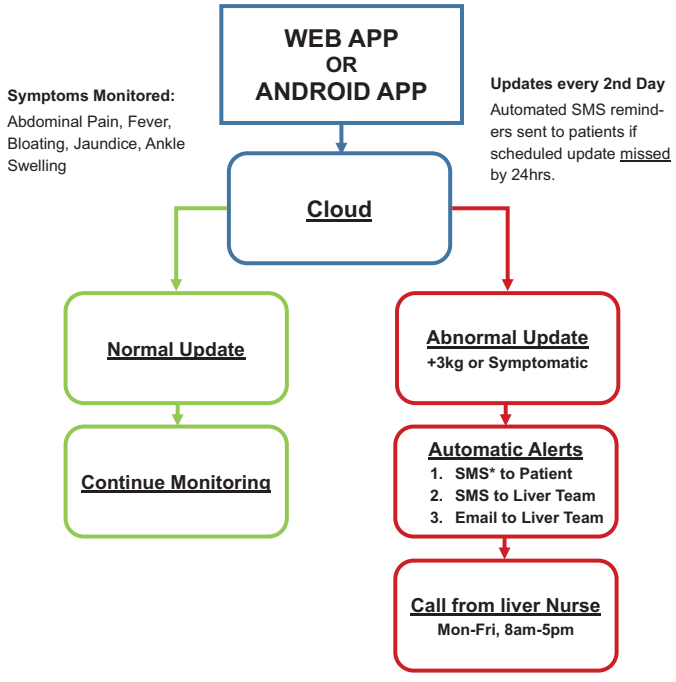

Figure 1.

P0196
NUTRITIONAL STATUS EVALUATION OF NONHOSPITALIZED PATIENTS WITH ALCOHOLIC LIVER CIRRHOSIS

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Background and Aims: Malnutrition in cirrhotic patients is common and the degree of nutrition debilitation can play an important role in the complications and cause a negative impact on prognosis. However, the assessment of the malnutrition in these patients is still a problem because liver disease can affect the results of many of the traditional techniques currently used to evaluate nutritional status. The best method of nutritional evaluation in cirrhotic patients remains controversial. Aim: To evaluate the frequency of malnutrition in outpatients with alcoholic cirrhosis (AC) using different assessment methods of nutritional state
Methods: Prospective study of AC outpatients (diagnosis based on histological evidence and/or high clinic, biochemical, imagiological suspicion). Exclusion criteria: malabsorption syndrome, chronic pancreatitis, inflammatory bowel disease, chronic kidney disease, acquired immunodeficiency syndrome, neuromuscular diseases and oncologic advanced disease. Included patients were classified according to the disease severity through Child–Pugh score. Nutritional state was determined by Subjective global assessment (SGA), Nutritional index (NRI), Maastricht index (MI), Instant nutritional assessment (INA), Mid arm circumference (MAC) and Triceps skinfold thickness (TST). Based on this evaluation patients were classified into the three groups: well, moderately and severely malnourished. Food intake was retrospectively evaluated using 24-hour dietary recall data.
Results: Include 80 AC patients, 60% were male and men age were 52.3±8.2 years. Concerning cirrhosis severity 51.3% were Child–Pugh A, 32.5% were B and 16.2% were C. Variable degrees of malnutrition were diagnosed: 12.5% patients by TST, 17.5% by MAC, 26.3% by SGA, 63.7% by NRI, 66.3% by INA and 81.3% by MI. MI detected malnutrition in early stages of hepatic disease with more sensibility and specificity than the others methods. The prevalence malnutrition increased according to the severity of AC. Fat reserves were more depleted in females than in males, whereas muscle reserves were more depleted in males. More advanced malnutrition was associated with lower energy and protein intake.

Conclusions: The diagnostic of malnutrition varies according to the nutritional evaluation method used. Traditional nutritional assessment, although easier, underestimated the prevalence and severity of malnutrition in patients with cirrhosis. The IM was the best single score to identify the patients who had malnutrition, including early stage.

P0197

THE THROMBOEMBOLIC RISK AMONG HOSPITALIZED CIRRHOTIC PATIENTS AND LOW-MOLECULAR-WEIGHT HEPARIN SAFETY

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Background and Aims: *Background:* The cirrhotic patient represents a unique subset of patients who are at risk for both bleeding and developing venous thromboembolic events (VTE). It has been commonly misunderstood that these patients are naturally protected from thrombosis by deficiencies in coagulation factors. As a result, the cirrhosis population is often falsely perceived to be "autoanticoagulated". While patients with cirrhosis may have a bleeding predisposition, not uncommonly they also experience thrombotic events.
Aim: The aim of our study is to evaluate the risk of VTE among cirrhotic patients admitted to the hospital compared with age-gender mach control group.
Methods: Retrospective study that enrolled all cirrhotic patients admitted to the internal medicine departments of the Holy Family Hospital at Nazareth, and Sharee Zedek Medical Center at Jerusalem between 2005and 2012, all cases of VTE were analyzed and charts were extracted to determine the incidence and risk factors of VTE among cirrhotic patients compared with control group.
Results: 792 admissions of cirrhotic patients were documented between 2005and 2012, and 500 admissions match age-gender control group. During this period among the cirrhotic patients were 5 documented new VTE events; 0.68% of all admissions of cirrhotic patients resulted in a new diagnosis of VTE event. Multivariate analysis shows that the severity of liver disease (MELD/Child), and laboratory parameters not affected the risk of VTE.
Regarding the control group 3 new VTE events were documented; 0.6% of all admissions of control group (P = NS).
Regarding the 5 events within the cirrhotic group, 3 (60%) were DVT, 1 (20%) was PE only, and 1 (20%) involved both PE and DVT. From the 4 cases of the DVT, 3 were proximal and one distal, from the 2 PEs, one was massive and the other segmental. The 3 case among the control group were one case of isolated DVT, one isolated PE, and one PE + DVT.
All patients with VTE were treated with LMWH according to the guidelines, no bleeding complication were documented in both groups.
Conclusions: The risk of VTE, among cirrhotic patients appears to be similar to other admitted patients. Traditional markers of coagulation such PT, INR, platelets count were not predictive for VTE. The DVT among cirrhotic may be predominantly proximal. The uses of LMWH appear to be safe in treatment cirrhotic patients diagnosed with VTE.

Cirrhosis and its complications: c. Bleeding, Hepatorenal syndrome and Ascites

P0198

PERCENTAGE CHANGE IN SERUM CREATININE (SCR) IS A SENSITIVE INDICATOR OF THERAPEUTIC RESPONSE TO TERLIPRESSIN IN HEPATORENAL SYNDROME TYPE 1 (HRS-1)

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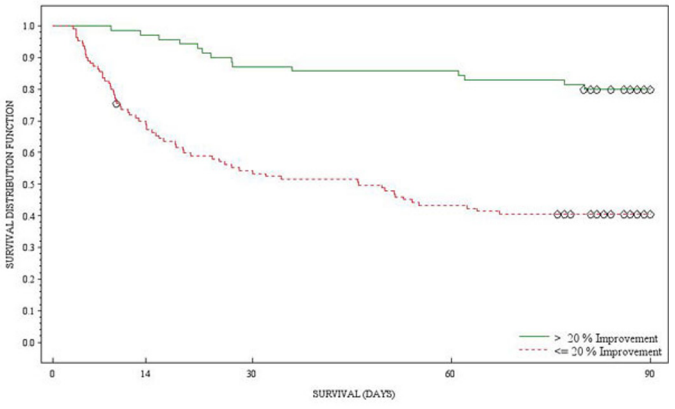
Background and Aims: The definition of response in most studies of terlipressin for HRS-1 has been HRS reversal, defined as a decrease of SCr to below 1.5 mg/dL, and partial response, a decrease in SCr by ≥50% but still ≥1.5 mg/dL. Recently, criteria for acute kidney injury (AKI) have focused on change in SCr rather than an absolute value to define AKI. The REVERSE study, the largest placebo controlled trial of terlipressin plus albumin vs. albumin alone for the treatment of HRS-1 has provided the opportunity to further examine improvement in SCr as a marker of response to treatment.
Methods: Subjects with HRS-1 were randomized to terlipressin (1 mg IV every 6 hours) or placebo, plus albumin in both groups. Endpoints evaluated included confirmed HRS reversal (CHRSR – 2 SCr values ≤1.5 mg/dL 48 hrs apart), HRS reversal, the percent change in SCr from baseline to the last day of dosing by 20% increments or decrements, overall survival, and survival at Day 90 without renal replacement therapy (RRT).



20% improvement is improvement from baseline to the last SCr on the date of last dose.
The p-value comparing the survival estimates is from a two-sample log-rank test stratified by qualifying Serum Creatinine and alcoholic hepatitis. P-Value= < 0.0001

Results: Linear regression analysis showed that survival is highly correlated with percent change in SCr (r2 = 0.9568; p < 0.0001). All subjects with CHRSR or HRS reversal had a >20% decrease in SCr from baseline; however, 38/70 (45.7%) subjects with >20% decrease in SCr did not achieve HRS reversal. The survival of subjects with >20% improvement in SCr was significantly higher than subjects with ≤20% improvement in SCr (p < 0.0001) (Figure). In addition, the survival of subjects with >20% improvement in SCr without CHRSR was similar to that seen when subjects with CHRSR were included and was significantly higher compared to subjects with ≤20% improvement (p < 0.0001). The number of subjects with >20% improvement in SCr with terlipressin (42/97, 43.3%) was significantly greater compared to placebo (28/99, 28.3%) (p < 0.05). In subjects with >20% improvement in SCr, 31/34 (91%) in the

terlipressin group were alive without RRT at Day 90 vs. 15/22 (68%) in the placebo group ($p < 0.05$).
Conclusions: These data show that a survival benefit occurs in subjects with HRS-1 with a >20% improvement in SCr from baseline even in the absence of HRS reversal. More subjects treated with terlipressin and albumin have a >20% decrease in SCr and have a better outcome at Day 90 compared to subjects treated with albumin alone. A percentage change in SCr from baseline may provide a more inclusive and sensitive indicator of response to terlipressin in HRS-1.

P0199
ACUTE KIDNEY INJURY IN CIRRHOSIS: BASELINE SERUM CREATININE PREDICTS PATIENT OUTCOMES

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Background and Aims: Various definitions of acute kidney injury (AKI) using different changes in renal function from baseline have recently been applied to determine prognosis in patients with cirrhosis. Reported patient outcome varies according to AKI definition. The International Ascites Club (IAC) has defined Stage 1 AKI in cirrhosis as an increase in serum creatinine (SCr) by ≥0.3 mg/dL in ≤48 hours, or ≥50% increase from a stable baseline SCr (B-SCr) in ≤3 months. It is unclear what influence B-SCr may have on post-AKI patient and renal outcomes.
Aim: To determine the association between B-SCr and the course of AKI and patient survival.
Methods: NACSELD (North American Consortium for the Study of End-Stage Liver Disease) is a consortium of tertiary-care hepatology centers that prospectively enrols non-elective cirrhotic inpatients. Patients with AKI as defined by IAC were included in the study. Course of AKI and 30-day survival were compared between patients with different levels of B-SCr (≤0.5 mg/dL, 0.51–1.0 mg/dL, 1.01–1.5 mg/dL, >1.5 mg/dL).
Results: 653 middle-aged (56.7±10 yrs old, 64% men) cirrhotic inpatients with mostly alcohol (26%) and hepatitis C (23%) induced cirrhosis were included. 30% were admitted with an infection, with equal frequency amongst groups ($p = 0.8$). Patients with the highest B-SCr more often had concomitant diabetes. 303 patients developed AKI, significantly more so in patients with B-SCr of >1.5 mg/dL, who also had a significantly greater increase in SCr ($p < 0.001$). AKI outcomes were defined as progressive (SCr worsened and ended in dialysis), persistent (SCr remained high) or transient (SCr returned to baseline). Significantly more patients developed progressive AKI, associated with a significantly decreased 30-day survival, as the B-SCr increases. Patients whose B-SCr was ≤1.0 mg/dl mostly had transient AKI with excellent 30-day survival (95%). Multivariate logistic regression shows that both B-SCr and ΔSCr during AKI are the only 2 significant factors impacting 30-day survival ($p < 0.001$).
Conclusions: Cirrhotic patients with pre-existing renal dysfunction, as indicated by a high B-SCr, are at higher risk for developing AKI, irrespective of admission indication. This risk increases with increasing B-SCr, and is strongly associated with an increased likelihood of AKI progression and death. Therefore, patients with high B-SCr may be considered a high-risk group for worst outcome.

Variable	B-SCr ≤0.50 mg/dL (n = 18)	B-SCr 0.51–1.00 mg/dL (n = 283)	B-SCr 1.01–1.50 mg/dL (n = 209)	B-SCr >1.50 mg/dL (n = 143)	p-value
B-SCr (SD)	0.45 (0.04)	0.77 (0.13)	1.18 (0.15)	2.46 (1.21)	<0.0001
Peak SCr (SD)	0.67 (0.12)	1.19 (0.75)	1.85 (1.05)	3.60 (1.82)	<0.0001
Diabetes	41%	27%	41%	48%	<0.0001
AKI (n = 303)	12%	26%	54%	69%	<0.0001
AKI outcome					
Progressive	0%	9%	11%	35%	
Persistent	0%	23%	36%	34%	
Transient	100%	69%	53%	32%	<0.0001
30-day survival	94%	95%	93%	75%	<0.0001

P0200
EARLY TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT IN CIRRHOTIC PATIENTS WITH ACUTE VARICEAL BLEEDING: A META-ANALYSIS OF CONTROLLED TRIALS

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Background and Aims: There is conflicting evidence regarding the benefit of early transjugular intrahepatic portosystemic shunt (TIPSS) on survival of cirrhotic patients with acute variceal bleeding (AVB). Aim: To assess the effect of early TIPSS on patient prognosis.
Methods: We performed a meta-analysis of controlled trials evaluating TIPSS performed within 72 hours of the initial endoscopy in cirrhotic patients with AVB treated with the standard-of-care.
Results: Four studies including 252 patients were included. Early TIPSS was associated with fewer deaths (OR = 0.38, 95% CI=0.17–0.83, $p = 0.02$). There was a moderate heterogeneity between studies ($p = 0.15$, I^2=44%). After exclusion of a study in which patients allocated to TIPSS were compared to historical control and which included patients with very severe liver disease, there was a significant effect of early TIPSS on mortality (OR = 0.26, 95% CI=0.13–0.51, $p < 0.001$) with no significant heterogeneity ($p = 0.9$, I^2=0%). Among Child–Pugh B patients, early TIPSS was not significantly associated with fewer deaths (OR = 0.35, 95% CI=0.10–1.17, $p = 0.087$). There was no heterogeneity between studies ($p = 0.6$, I^2=0%). Among Child–Pugh C patients, early TIPSS was not significantly associated with fewer deaths (OR = 0.34, 95% CI=0.10–1.11, $p = 0.074$). There was a high heterogeneity between studies ($p = 0.06$, I^2=60%). After exclusion of a study in which patients allocated to TIPSS were compared to historical control, there was a significant effect of early TIPSS on mortality (OR = 0.21, 95% CI=0.07–0.66, $p = 0.007$) with less heterogeneity ($p = 0.22$, I^2=33%). Early TIPSS was also associated with better 6-week (OR = 3.03, 95% CI=1.41–6.49, $p = 0.004$) and 1-year (OR = 2.42, 95% CI=1.03–5.67, $p = 0.04$) survivals. Less patients treated with early TIPSS reached the composite endpoint defined as failure to control bleeding or failure to prevent clinically significant bleeding within one year (OR = 0.08, 95% CI=0.04–0.17, $p < 0.001$), with no heterogeneity between studies ($p = 0.7$, I^2=0%). Results were similar among Child–Pugh B (OR = 0.15, 95% CI=0.05–0.47, $p = 0.001$) and Child–Pugh C patients (OR = 0.05, 95% CI=0.02–0.15, $p < 0.001$). Early TIPSS was not associated with higher rates of encephalopathy (OR = 0.84, 95% CI=0.50–1.42, $p = 0.5$).
Conclusions: Cirrhotic patients with AVB treated with early TIPSS had lower death rates and improved 6-week and 1-year survivals as compared to patients treated without early TIPSS. Child–Pugh C patients with AVB may also benefit from early TIPSS.

P0201

OUTCOMES AND PREDICTORS OF SURVIVAL IN PATIENTS WITH ACUTE-ON-CHRONIC LIVER FAILURE AND SUPERIMPOSED ACUTE KIDNEY INJURY REQUIRING DIALYSIS

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Background and Aims: Acute-on chronic liver failure (ACLF), a heterogeneous clinical entity is defined as acute hepatic insult, manifesting as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease. It is often complicated with acute kidney injury (AKI) due to the underlying hemodynamic alterations. Patients with ACLF, especially with a superimposed AKI have higher morbidity and mortality. Apart from conventional hemodialysis (HD) or continuous renal replacement therapies (CRRT), hybrid therapies like Sustained low efficiency dialysis (SLED) are often used in this setting. There is paucity of literature to help identify patients who will benefit more with RRT than others and patient outcomes and predictors of survival in this setting are not completely known.
Methods: We conducted a prospective observational study between May 2009 and September 2013 and followed 170 patients having an admission diagnosis of ACLF and had AKI at any time during the hospitalization. A diagnosis of ACLF was made as per the 2008 guidelines of Asian Pacific Association for the study of the liver (APASL) ACLF working group. Their clinical courses, requirement of RRT and laboratory parameters were noted. Conventional HD and SLED were the only RRT modalities available.
Results: Out of a total of 170 patients, 117 were provided RRT (conventional HD or SLED). The mean MELD and SOFA scores were 42.3 and 12.8 respectively. SLED using a femoral venous catheter was the commonest modality used. 53% of all patients had intra-dialytic complication, most common being hypotension. The mean survival after the initiation of RRT was 11.6±2.02 days. A multivariate analysis (Table 2) showed that higher CTP score (mean 12.5 versus 10.5, $p < 0.001$), higher SOFA score (mean 7.1 versus 14.1, $p < 0.001$), higher mean MELD score and a lower serum creatinine level were independent predictors of mortality.

Table: Multivariate analysis of clinical and laboratory features of survivors and non-survivors

	ACLF survived on RRT	ACLF died on RRT	P value
Acute onset ascites	33.3%	94.4%	**<0.001**
Hepatic encephalopathy	66.6%	96.6%	**<0.001**
MELD score	40	43	**0.02**
CTP score	10.5	12	**<0.001**
Total bilirubin	21	33.5	**<0.001**
INR	2.2	3.04	**0.003**
Serum albumin	2.3	2.4	0.5
Mean blood urea	120.8	175.1	**<0.001**
Mean serum creatinine	4.3	3.5	**0.01**
Mean SOFA score	7.8	14.1	**<0.001**
Inotrope requirement			**<0.001**
1. Low dose	25%	0	
2. Moderate dose	62.5%	2.2%	
3. High dose	0	37.7%	
4. Double/triple inotrope	0.4%	0.4%	
Complication on RRT			
1. Hypotension	0	43.7%	**<0.001**
2. Cardiac arrhythmia	4.1%	18.8%	

Conclusions: ACLF patients with AKI requiring RRT (HD and SLED) have a very high mortality. Higher CTP, SOFA, MELD scores along with lower serum creatinine levels (probably signifying sarcopenia and lower creatinine generation) are independently associated with a higher mortality. Large prospective trials are necessary to select appropriate patients who would benefit most with RRT in addition to defining appropriate mode, timing and dose of RRT.

P0202

PLACEMENT OF A PERMANENT, TUNNELLED PERITONEAL DRAINAGE CATHETER (PLEURX) FOR REFRACTORY MALIGNANT AND PORTAL-HYPERTENSIVE ASCITES IN A MULTICENTRE STUDY

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Background and Aims: Repeated large-volume paracentesis is the best-established treatment option in malignant ascites and in treatment refractory portal-hypertensive ascites with contraindications to TIPS. However, rapid reaccumulation of ascites requires frequent hospital visits and punctures in this frail patient population. A one-time implantation of a permanent tunnelled catheter is frequently used for treatment of malignant pleural effusions. Our aim was to study feasibility and safety of this easy to use home drainage system in patients with refractory ascites.
Methods: Retrospective analysis from three Swiss tertiary centers after subcutaneous implantation of a 15.5 French peritoneal catheter (PleurX®, CareFusion) under conscious sedation (propofol) and antibiotic prophylaxis (ceftriaxone i.v. 30 min before procedure) by trained gastroenterologists in patients with refractory ascites and contraindication to TIPS. Patients received instructions on handling of drainage bottles to perform home based paracentesis through the PleurX catheter as needed.
Results: Between 03/2013 and 11/2014, 32 PleurX® catheters were implanted in 32 patients [age 29–77, mean age 55, 15/32 (47%) women]. Indications were malignant ascites (23 patients), portal-hypertensive ascites with (3 patients) and without (6 patients) advanced HCC. Success rate for catheter implantation was 97% (31/32) with 1 failure due to peritoneal cancer infiltration. PleurX® insertion lead to immediate symptom relief without the need of additional catheter intervention or separate therapeutic paracentesis after catheter placement. No procedure-related deaths or major placement complications occurred. No serious adverse events were reported. Three cases of bacterial peritonitis (2 in HCC patients, 1 in malignant ascites without portal hypertension) were successfully treated with antibiotics. Twenty-one patients died during follow up due to disease progression (3–227 days after intervention, mean 38 days). Catheter survival ranged from 3 to 291 days (mean 64 days, 1,977 cumulative catheter-days). Four catheters were removed 17–143 days after implantation due to liver transplantation (n = 1), resolution of ascites (n = 2) and local infection (n = 1).
Conclusions: Placement of a permanent, tunnelled peritoneal drainage catheter is an easy to perform and safe alternative to repeated paracentesis in selected patients with refractory ascites.

P0203

SOLUTE-FREE WATER RETENTION IN EXPERIMENTAL ASCITIC CIRRHOSIS: VASOPRESSIN (ADH) IS NOT THE ONLY AGENT TO BLAME

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Background and Aims: Increased adrenergic function causes proximal tubular fluid retention and reduces renal excretion of solute-free water, but, in advanced cirrhosis, non-osmotic hypersecretion of vasopressin (ADH) is considered the cause of water retention and hyponatremia. Aim. Since ADH V2 receptor

antagonists are not beneficial in long-term treatment of ascites, we hypothesize that water retention in experimental ascitic cirrhosis depend also on adrenergic hyperfunction.
Methods: Hormonal status, renal function and tubular free-water reabsorption (TFWR) were assessed in 6 groups of rats with ascitic cirrhosis: rats with cirrhosis due to 13-week CCl_4 administration (group G1); cirrhotic rats receiving daily diuretics (0.5 mg/kg furosemide plus 2 mg/kg K+-canrenoate) during 11th-13th weeks of CCl_4 (G2); cirrhotic rats treated daily with diuretics associated with guanfacine oral prodrug (α2A adrenergic receptor agonist and sympatholytic agent) 2 (G3), 7 (G4), or 10 mg/kg (G5), or SSP-004240F1 (V2 receptor antagonist) 1 mg/kg (G6) during 11th-13th weeks of CCl_4.
Results: Sodium excretion was lower in G1 than G2–G4 and G6 (all $P < 0.05$). TFWR was higher in G1 (untreated cirrhosis, 32±11 microL/min) than in G6 (diuretics + V2-antagonists, 21±9 microL/min) or G3 (diuretics + guanfacine 2 mg/kg, 20±8 microL/min) (all $P < 0.03$). Guanfacine, added in G3 to sole diuretics (G2), reduced serum norepinephrine from 423±122 to 211±111 ng/L ($P < 0.01$), plasma renin activity from 25±12 to 9±7 ng/mL/h ($P < 0.03$), and TFWR from 45±18 to 20±8 microL/min ($P < 0.01$). Among all rats, TFWR did not correlate with ADH levels ($r = 0.02$, P: n.s.), but did with plasma aldosterone ($r = 0.51$, $P < 0.01$) and urinary potassium excretion ($r = 0.90$, $P < 0.001$).
Conclusions: In ascitic cirrhosis, reduced volaemia, adrenergic hypertone, and secondary aldosteronism, especially when exacerbated by loop diuretics, cause tubular retention of water. Sympatholytic agents are as effective as V_2-antagonists to blunt tubular free water retention.

P0204
NONINVASIVE PREDICTION OF HEMODYNAMIC RESPONSE TO BETA-BLOCKER THERAPY IN CIRRHOTIC PATIENTS WITH ESOPHAGEAL VARICES

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Background and Aims: Nonselective beta-blocker (NSBB) is a mainstay of both primary and secondary prophylaxis of esophageal variceal bleeding in cirrhotic patients. Although hepatic venous pressure gradient (HVPG) is an essential method to evaluate hemodynamic response to NSBB, measuring HVPG is invasive and requires special facility and expertise. The aim was to investigate whether noninvasive markers of portal hypertension correlate with hemodynamic response to NSBB in cirrhotic patients with esophageal varices.
Methods: In this prospective study, 160 cirrhotic patients with esophageal varices who were indicated for primary or secondary prophylaxis of hemorrhage were enrolled between May 2013 and July 2014. As of October 2014, 76 patients completed paired measurements of HVPG, liver stiffness (LS) and spleen stiffness (SS) both at the beginning and at the end of dose titration (maximal tolerated dose) of carvedilol therapy. LS and SS were measured using acoustic radiation force impulse imaging.
Results: Mean age was 59 years, and male patients were 55 (72.4%). Main etiologies of cirrhosis were viral [HBV, 25 (32.9%); HCV, 9 (11.8%)] and alcoholic [32 (42.1%)]. Primary and secondary prophylaxes were done in 41 (53.9%) and 35 (46.1%), respectively. Size of varices were F2 or 3 in 57 (75%) and red color sign was shown in 36 (47.4%). Child–Pugh class was mostly A (38, 50%) or B (27, 35.5%). Baseline HVPG was 19 mmHg (12–39). Baseline median LS and SS were 2.45 m/s and 3.13 m/s, respectively. Hemodynamic response (decrease in HVPG ≥20% from baseline or to absolute value <12 mmHg) was achieved in 42 (55.3%) on a median 25 mg of carvedilol. Responders showed 7.2 mmHg mean decline of HVPG from their baseline level. Both LS values at baseline (LS1, 2.29 vs. 2.63 m/s; $P = 0.037$) and at the end of dose titration (LS2, 2.13 vs. 2.58 m/s; $P < 0.001$) were significantly lower in responders than in nonresponders. SS values did not show differences between responders and nonresponders. Areas under the receiver operating curves for predicting hemodynamic response were 0.665 for LS1 and 0.737 for LS2, respectively.
Conclusions: Liver stiffness at baseline and at the end of dose titration may be predictive of hemodynamic response to carvedilol therapy as primary or secondary prophylaxis for hemorrhage in cirrhotic patients with esophageal varices.

P0205
MANAGEMENT OF GASTRIC VARICES: A FRENCH NATIONAL SURVEY

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Background and Aims: Gastric variceal bleeding accounts for 10% of upper gastrointestinal bleeding related to portal hypertension. Level of evidence in managing gastric varices is low. We aimed to determine the modalities of management of non-GOV1 gastric varices in France.
Methods: Hepato-gastroenterologists (HGE) working in general hospitals (GH) or in university hospitals (UH) received a self-administered questionnaire.
Results: One hundred and fifty-four HGE from 109 centers (37 UH, 72 GH) among the 336 (32.4%) contacted responded. Regarding primary prophylaxis, beta-blockers were used by 96% of HGE. Only 17.2% of HGE used glue obliteration (UH: 27.7% *vs* GH: 9.3%; $p = 0.004$), 8% used TIPS and 5.3% proposed no treatment. Most HGE (77.6%) estimated that they had local access to glue obliteration but 64.2% declared that TIPS placement required transfer to another center. Obliteration was performed under general anesthesia by 86% of HGE. N-butyl-2-cyanoacrylate plus methacryloxysulfolane (Glubran®) and N-butyl-2-cyanoacrylate (Histoacryl®) were used respectively in 48.2% and 55.9% of cases (3.5% of HGE used both). Dilution with lipiodol was performed in 78% of cases. The technique of obliteration was variable between centers and within the same center: maximal dilution was 1/10; injected volume varied from 0.5 to 20 mL per varix and from 1 to 30 mL per procedure. To control active bleeding, 77.6% of HGE used obliteration (UH: 85.7% vs GH: 70.9%, $p = 0.04$) and 34% used band ligation (UH: 28.6% vs GH: 38.8%; $p = 0.02$). Early-TIPS was proposed by 56.3% of HGE (UH: 71.7% vs GH: 39.2%, $p < 0.001$). Regarding secondary prophylaxis, 74.4% used beta-blockers, 66% used obliteration (UH: 76.6% vs GH: 56.6%; $p = 0.014$) and 14% used TIPS. Endoscopic control was performed by 62.6% of HGE, 70% evaluated the varix stiffness with a closed biopsy forceps. Side effects of obliteration were reported by 59.5% of HGE (UH: 70.4% vs GH: 41.2%; $p = 0.08$) and concerned mainly glue migration (UH: 66.7% vs GH: 33.3%; $p < 0.001$), an event systematically searched by 22.9%.
Conclusions: The management of gastric varices in France is very heterogeneous between centers and even within the same center. University hospitals have a better access to obliteration and especially to TIPS. Obliteration as a primary prophylaxis procedure was rarely performed. Glue migration was frequently

observed although probably underreported. Specific guidelines on the management of gastric varices should be established by expert groups to standardize clinical practices.

P0206

PREVENTION OF BLEEDING FOLLOWING INVASIVE PROCEDURES IN CIRRHOTIC PATIENTS: A SINGLE CENTER EXPERIENCE

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Background and Aims: Risk of bleeding in cirrhosis has predominantly been associated with coagulopathy and thrombocytopenia due to impaired liver function and splenomegaly. The evaluation of the actual bleeding risk in cirrhotic patients undergoing invasive procedures is a critical point to optimize management in terms of platelet (Plt) or plasma prophylactic transfusions.
Methods: From January 2013 to December 2013, 480 cirrhotic patients underwent diagnostic or therapeutic invasive procedures at our Liver Unit (Fondazione IRCCS Ca Granda, Ospedale Maggiore Policlinico, Milan, Italy). Per protocol, Plt transfusion was deemed necessary for Plt count less than 50,000/mmc while fresh frozen plasma was infused to patients with international normalized ratio (INR) >1.5, except those undergoing paracentesis. Low-molecular-weight-heparin was discontinued 24 hours before any procedure while antiaggregant therapy was interrupted five days before any procedure except oesophageal varices band ligation and paracentesis. Major hemorrhagic events were those requiring hospitalisation or blood transfusion; minor events was a haemoglobin decline >1.5 g/dl post procedure not requiring hospitalization and without bleeding evidence.
Results: 174 Transarterial Chemo Embolization (TACE), 16 Radio-Frequency Termal Alblation (RFTA), 214 paracentesis, 59 oesophageal varices banding, 6 trans-jugular liver biopsy (LB) and 11 Percutaneous Ethanol Injection (PEI), were performed. Overall, 61 procedures met the criteria for plasma or Plt infusion and major bleeding complications occurred in 3 patients (0.6%). In 2 patients, anemia-related paracentesis was treated by blood transfusions whereas one patient following variceal band ligation, had to be hospitalized for severe anemia. Major complications rate was 1.6% in Plt/plasma infusion exposed patients versus 0.47% in unexposed patients (p = 0.28). Minor events with a >1.5 g/dl haemoglobin decline occurred in 17 patients (15 paracentesis and 2 band ligations), the rate was 3.2% in Plt/plasma infusion exposed patients versus 3.5% in unexposed (p = 0.9).
Conclusions: Pre-treatment platelet transfusion in cirrhotic patients with Plt count less than 50,000/mmc and plasma infusion for those with INR >1.5, was associated with a negligible risk of bleeding and appeared as a safe, cost/effective strategy.

P0207

GUANFACINE (SPECIFIC ALPHA-2A ADRENOCEPTOR AGONIST) RESTORES NATRIURESIS IN EXPERIMENTAL CIRRHOTIC ASCITES RESISTANT TO CLONIDINE AND STANDARD DIURETICS

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Background and Aims: In human cirrhosis, adrenergic hyperfunction causes proximal tubular fluid retention and contributes to refractory ascites; clonidine, a sympatholytic drug, plus diuretics improve natriuresis in otherwise refractory ascites.
Aim: To compare clonidine (aspecific α2-adrenoceptor agonist) and SSP-002021R (specific α2A-receptor agonist and prodrug of guanfacine), both associated with diuretics, in cirrhotic refractory ascites.
Methods: Seven groups of 12 rats were studied: controls (G1); controls receiving furosemide and potassium canrenoate (G2); rats with ascitic cirrhosis due to 14 CCl_4 weeks (G3); cirrhotic rats treated with furosemide and canrenoate over the 11th–14th CCl_4 weeks (G4); cirrhotic rats treated with furosemide, canrenoate and clonidine (0.5 mcg) (G5); cirrhotic rats treated with diuretics and low-dose clonidine (0.3 mcg) (G6); cirrhotic rats treated with diuretics and SSP002021R (5 mg/kg b.w.) (G7). Three rats in each group had their hormonal status and renal function assessed at the end of 11th, 12th, 13th, and 14th CCl_4 weeks.
Results: Cirrhotic rats in G3 and G4 gained weight over the 11th–14th CCl_4 weeks. In G4, brief increase in sodium excretion due to diuretics (12th week) preceded worsening of inulin clearance and natriuresis (diuretic resistance). The addition of low-dose clonidine (G6) or guanfacine (G7) to diuretics increased, respectively, electrolytes excretion over the 11th–12th CCl_4 weeks, or GFR and electrolytes excretion over the 13th–14th CCl_4 weeks. Natriuretic responses in G6 and G7 were accompanied by significantly reduced catecholamines' serum levels vs. G3 and G4 (all $P < 0.03$).
Conclusions: Clonidine briefly potentiates diuretics-dependent natriuresis. Guanfacine (α2A-receptor agonist) restore glomerular filtration rate and natriuresis, and prevent refractory ascites in experimental advanced cirrhosis.

P0208

HEMODYNAMIC RESPONSE TO NON-SELECTIVE BETA-BLOCKERS IS NOT INFLUENCED BY THE PRESENCE OF METABOLIC SYNDROME

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Background and Aims: Insulin resistance and the metabolic syndrome have been associated with the severity of portal hypertension (PHT) in patients with cirrhosis. Response to non-selective betablockers (NSBBs) is evaluated by sequential measurements of the hepatic venous pressure gradient (HVPG), and defined as complete response (CR: decrease of HVPG ≥20% or to absolute values <12 mmHg), partial response (PR: HVPG decrease 10–<20%), or nonresponse (NR: HVPG decrease <10%). We aimed to assess the relationship between metabolic syndrome (MS) and hemodynamic response rate to NSBBs in patients with cirrhosis.
Methods: We retrospectively included patients with paired HVPG measurements. MS was diagnosed by the International Diabetes Federation criteria in paients with obesity (body mass index – BMI >30 kg/m^2) presenting with at least two of the following critiria: (1) elevated triglycerides >150 mg/dL; (2) reduced HDL cholesterol <40 mg/dL in males or <50 mg/dL in females; (3) arterial hypertension: systolic BP >130 mmHg or diastolic BP >85 mmHg; (4) elevated fasting plasma glucose >100 mg/dL, or previously diagnosed type 2 diabetes. Patients with BMI >30 kg/m^2 due to grade II/III ascites were not considered as having MS and were excluded (n = 5).
Results: 278 patients with paired HVPG measurements were included (55.1% propranolol, 44.9% carvedilol). MS was diagnosed in 11.1% (31/278 patients), the proportion of patients treated with propranolol and carvedilol was similar in patients with MS (44.9% vs. 45.2%, p = 0.87) as well as the doses of NSBBs (p = 0.72

for propranolol, and p = 0.45 for carvedilol). Overall 46.8%, 16.5%, and 36.7% of patients had CR, PR, and NR, respectively. The baseline HVPG (mmHg) was similar in patients without and with MS: 20.1±4.6 vs. 18.8±4.3 (p = 0.16). The median HVPG reduction was similar in patients without and with MS: −15.7% (IQR: −3.73 to −27.7) vs. −17.3% (IQR: +6.07 to −25; p = 0.26). The distribution of NSBB response was comparable between patients without or with MS: CR: 46.9% vs. 45.2% (p = 0.99); PR: 17% vs. 12.9% (p = 0.74); and NR: 36.1% vs. 41.9% (p = 0.66).
Conclusions: The hemodynamic response rate to NSBBs in cirrhotic patients with PHT is not influenced by the presence of the metabolic syndrome.

P0209
A COMMON POLYMORPHISM OF TUMOR NECROSIS FACTOR RECEPTOR-ASSOCIATED FACTOR 6 (TRAF6) IS ASSOCIATED WITH AN INCREASED RISK FOR SPONTANEOUS BACTERIAL PERITONITIS

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Background and Aims: Spontaneous bacterial infection (SBP) is a frequent complication of decompensated cirrhosis with high mortality. Monocytes and peritoneal macrophages are cellular immune regulators exhibiting pattern-recognition-receptors (PRR) to detect bacterial infections. We hypothesized that genetic polymorphisms of tumor necrosis factor receptor-associated factor 6 (TRAF6) are associated with an increased risk of SBP by modulating monocytic immune responses in patients with decompensated cirrhosis.
Methods: TRAF6 haplotype-tagging single nucleotide polymorphisms (SNPs) rs331457 (G/A) and rs5030419 (C/G) were determined by Fluorescence Resonance Energy Transfer/ Melt Curve analysis in 404 prospectively characterized hospitalized patients with decompensated cirrhosis. Episodes of SBP were identified from electronic records. We investigated mRNA expression of regulated genes by qRT-PCR in immunomagnetically isolated monocytes and peritoneal macrophages.
Results: The minor allele frequency for rs331457 was 16.5% and for rs5030419 was 14.4%. 193 (48%) presented with the TRAF6 wild-type (GGCC), 113 (28%) patients were carriers of the rs5030419 mutation (AGCG/GGCG/GGGG) and 98 (24%) patients were carriers of a haplotype with the rs5030419 wild-type and the rs331457 mutation (AGCC/AACC). 117 (29%) patients presented with at least one episode of SBP. Patients carrying the AGCC/AACC genotype had an increased risk of SBP (OR 1.93; 95% CI 1.20–3.12; p = 0.007) compared to patients not carrying this variant. Peritoneal macrophages from genotypically identified patients at risk demonstrated a lower expression of the neutrophil recruiting chemokine CXCL8.
Conclusions: A common variant in TLR signaling is associated with an increased risk of SBP in patients with decompensated cirrhosis, which may be employed for genotype-based primary prophylaxis.

P0210
COMBINED PARTIAL SPLENIC EMBOLIZATION (PSE) ALLOWS REDUCING TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT (TIPS) DIAMETER IN HYPERSPLENIC CIRRHOTIC PATIENTS: A PILOT STUDY

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Background and Aims: Portal hypertension (PHT) results from increased hepatic resistance and splanchnic volume. TIPS reduces hepatic resistance but worsen the hemodynamic stress. Such effect can be detrimental for the effectiveness of TIPS on PHT and promotes cardiac complications. Hypersplenism affect 40% of TIPS patients. The combination of PSE and TIPS by reducing the splenic venous backflow should decrease splanchnic volume and pressure. Furthermore, it may allow the creation of smaller calibre TIPS thus reducing the risk of encephalopathy. Additional expected beneficial effects of PSE are reduction of thrombocytopenia and an improved hepatic arterial perfusion.
Aim of our study was to evaluate the efficacy on PHT and safety of a combined procedure of PSE and TIPS in hypersplenic and cirrhotic patients candidates for a TIPS.
Methods: 14 cirrhotic patients with hypersplenism (defined by a platelet count <100,000/mm^3) gave their consent for a combined technique. Indication for TIPS was prevention of variceal re-bleeding prevention (n = 5), refractory ascites (n = 5), both (n = 2) and before surgery (n = 2). 8 patients were CHILD B, 4 were CHILD C and 2 Child A. Combined PSE was applied to allow further invasive procedures such as liver or orthopaedic surgery. The technique consisted in a transjugular portal catheterization with measurement of hepatic venous pressure gradient (HVPG) before and after PSE and after TIPS. PSE was performed by a non-selective injection in the splenic artery hilum of 1.5 to 3 vials of 900–1200 μm Embospheres®. TIPS was then performed by using Viatorr® stents, calibrated to obtain a HVPG <12 mmHg.
Results: After PSE, HVPG fell from 15.6±4.5 mmHg to 11.5±4.2 mmHg. After TIPS, HVPG fell to 6.2±2.5 mmHg representing a 59% reduction. In 8/14 procedures, TIPS could be undersized at 6 mm (57%). No complication occurred due to PSE.
Thirteen patients (93%) had no complication after a mean 4-months follow-up (±4).
Platelets count increased from 59±22 g/L to 248±113 one month after procedure.
One patient died from hepatic encephalopathy five months later.
Conclusions: This combined therapy, effective on the two components of PHT, has been shown as effective and safe in our pilot study, allowing the realization of smaller calibre TIPS. A randomized controlled study is needed to evaluate the other possible benefits of this approach on encephalopathy, liver function and cardiac complications of splanchnic hemodynamic stress in TIPS patients with hypersplenism.

P0211
HUMAN MICROFIBRIL-ASSOCIATED PROTEIN 4 (MFAP4) IN ASCITES FLUID PREDICTS SURVIVAL AND RISK OF COMPLICATIONS IN PATIENTS WITH ADVANCED CIRRHOSIS

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Background and Aims: Patients with cirrhosis and ascites are at high risk of death and development of complications to cirrhosis. Biomarkers to make a better risk stratification of these patients are warranted. We therefore investigated two biomarkers in ascites: (1) Human microfibril-associated protein 4 (MFAP4), an extracellular matrix (ECM) associated protein related to ECM turnover, and (2) Surfactant protein D (SP-D) which has a protective role in the innate immune system.
Methods: From 2011 to 2013 we prospectively enrolled 54 patients that were followed for 12 months or until death. The outcome evaluated where death, hepatorenal syndrome, hepatic encephalopathy, spontaneous bacterial peritonitis (SBP), non-SBP infections and readmissions. Eligible patients were patients with

verified cirrhosis, who had undergone therapeutic or diagnostic paracentesis. Patients who had been treated with antibiotics within the prior week and patients with malignant ascites were excluded. MFAP4 and SP-D where measured by ELISA technique.

Results: Characteristics of the enrolled patients were: Age 60.5±10.2 years, males/females 37/17, Child–Pugh-score (CP-score) 10.5±1.8 (CP A/B/C = 0/15/39), MELD-score 11.9±2.2. Four patients had SBP at inclusion. MFAP4 and SP-D in ascites were 20.6±10.3 U/ml (range 6.5–55.9) and 310.8±222.8 ng/ml (range 22–1212), respectively. In this cohort neither CP- nor MELD-score were predictors of death or risk of complications. However, in patients with low MFAP4 (≤15 U/ml), 8/16 patients died compared to 10/37 in the group with MFAP4 >15 U/ml (incidence-rate comparison P = 0.079). Among patients with low MFAP4, 13/16 versus 18/37 patients with MFAP4 >15 U/ml developed complications to cirrhosis (incidence-rate comparison P < 0.001), Figure 1. In a Cox proportional hazard model including CP- and MELD-score, age, gender, SP-D and MFAP4, low-MFAP4 (hazard ratio 4.72, CI 1.82–12.26, P = 0.001) and gender (HR 0.18, CI 0.05–0.65, P = 0.009) were the only predictors of complications. Of the four patients with SBP at inclusion, three had low MFAP4 and two died. SP-D was not able to predict survival, nor was it associated with risk of complications.

Conclusions: Low MFAP4 in ascites predicts individual 1-year survival and risk of complications in patients with advanced cirrhosis and ascites better than standard scoring systems as CP- and MELD-score. Further investigations are warranted to confirm the prognostic accuracy of MFAP4.

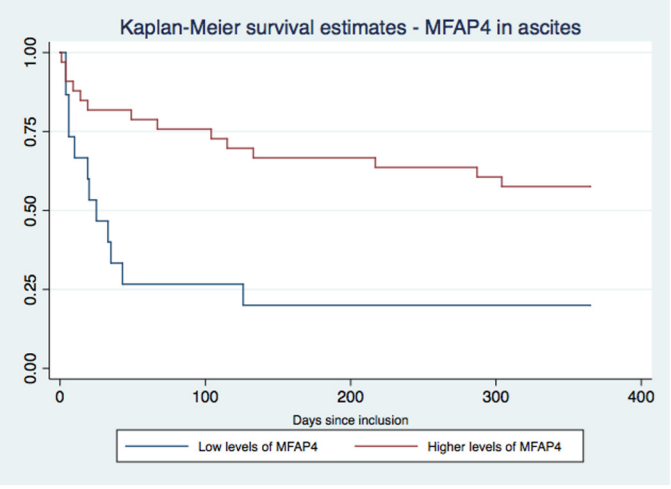

Figure 1.

P0212

IDENTIFYING THE TRIGGER MATTERS – OUTCOME OF SEVERE ACUTE KIDNEY INJURY IN PATIENTS WITH CIRRHOSIS AND ASCITES

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Background and Aims: Renal failure (RF) is a common cause of death in patients with cirrhosis and ascites. Acute kidney injury (AKI) can be triggered by complications that are highly prevalent in these patients, whereas diagnosis of hepatorenal syndrome (HRS), a special form of AKI, requires the exclusion of certain triggers. We aimed to investigate the impact of several triggers of severe AKI (grades 2+3) on transplant-free survival (TFS) and if TFS changes depending on whether the criteria for HRS were met or not.

Methods: Patients with cirrhosis and portal-hypertensive ascites who underwent paracentesis at the Medical University of Vienna were included in this retrospective study and their first episode of AKI or HRS was documented. Severe AKI was defined as an increase in serum creatinine (SCr) either of ≥100%, or ≥0.5 mg/dL to ≥4 mg/dL SCr within 48 h, or anuria, or renal replacement therapy. HRS was diagnosed either by an increase of ≥100% to ≥2.5 mg/dL SCr within 14 days or an increase from 1.5 mg/dL SCr in the absence of renal parenchymal lesions, proteinuria ≥500 mg/L, hematuria ≥50/HPF, nephrotoxic drug intake, severe hypovolemia/shock, significant cardiovascular disease, or severe trauma. AKI was further stratified by trigger. Survival was assessed using the Kaplan–Meier-Method and group comparisons performed using the log-rank test.

Results: 497 patients were included. A total of 148 episodes of severe AKI and 60 cases of HRS were detected. Median TFS of the AKI group was comparable to the HRS group: 19 (IQR 3–386) days vs. 17 (3–223) days, respectively (p = 0.656). There were no significant differences between these groups regarding patient characteristics. However, TFS varied depending on the trigger: Median TFS was only 7 (6–109) days in severe AKI triggered by nephrotoxins/surgery (n = 10) and 14 (3–312) days after severe hypovolemia/shock (n = 79). With 139 days (10-end of FU), median TFS was longest when occurring on top of chronic kidney disease and/or cardiovascular disease (n = 12). Median TFS of untriggered RF (n = 18) was 42 (9–1455) days.

Conclusions: Outcome of severe AKI and HRS was similar in our patient cohort with cirrhosis and ascites, questioning the utility in making a distinction based on the above definitions. Yet TFS varied depending on the trigger, suggesting a classification on the distinct mechanism/trigger could be clinically relevant. Prospective studies are warranted to confirm these data and investigate the efficacy of therapeutic interventions for AKI depending on the underlying trigger.

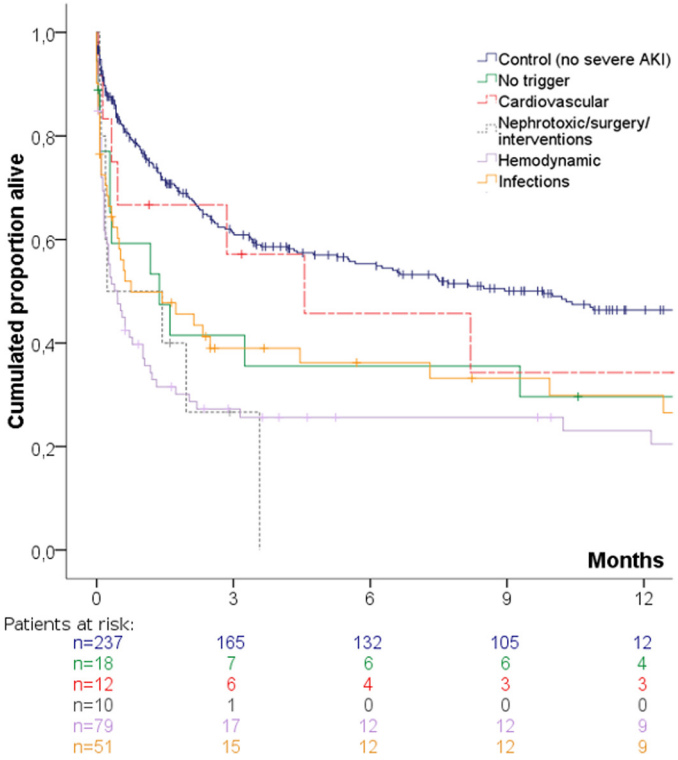

Figure: Transplant free survival of patients with cirrhosis and severe AKI according to trigger.

P0213

DIABETES IS A MAJOR DETERMINANT FOR REFRACTORY ASCITES IN PATIENTS WITH CIRHOSIS AND IS ASSOCIATED WITH HEPATIC MICROCIRCULATORY CHANGES

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Background and Aims: The causal factors for refractory ascites are poorly understood. Diabetes is associated with the development of ascites. The aims of this study were (1) to clarify the association of diabetes with refractory ascites in patients with cirrhosis; and (2) to provide insight into the mechanisms of refractory ascites by performing a comprehensive analysis of liver microscopic hepatic lesions associated with refractory ascites.
Methods: Consecutive patients with cirrhosis who underwent liver transplantation were retrospectively included. Univariate and binary multivariate logistic regression analysis of patients' characteristics at the time of listing were performed according to the presence or absence of refractory ascites. A comprehensive histological analysis of explanted native livers of the included patients was performed to determine lesions associated with refractory ascites.
Results: Eighty-five patients were included (78% men, mean age 51 years), including 30 with refractory ascites and 55 without (34 patients without ascites and 21 patients with diuretic-sensitive ascites). MELD score was higher (25±6.1 *vs.* 19.9±6.0, $p = 0.001$), and serum sodium was lower (133.0±6.7 *vs.* 138±3.3 mmol/L, $p < 0.001$) in patients with refractory ascites than in those without. Alcohol was more frequently a cause of liver disease (80% vs. 42%, $p = 0.001$) in patients with refractory ascites. Diabetes was more frequently observed (57% *vs.* 27%, $p = 0.008$) and hemoglobin A1c levels were higher in patients with refractory ascites than in those without (6.5±2.7% vs. 4.5±1.1%, $p = 0.006$). By multivariate analysis, refractory ascites was independently associated with a higher MELD score ($p = 0.005$), a lower serum sodium ($p = 0.005$), alcohol as a cause of cirrhosis (0.048), and diabetes mellitus ($p = 0.002$). Neither the amount of fibrosis, nor features of non-alcoholic fatty liver disease (macrovesicular steatosis, hepatocyte ballooning and Mallory bodies) differed in the two groups. By contrast, microvascular changes, namely sinusoidal dilatation ($p < 0.001$) and perisinusoidal fibrosis ($p = 0.001$), were more frequently observed in the livers of patients with refractory ascites.
Conclusions: Diabetes is associated with refractory ascites, independently of MELD score. Microscopic vascular lesions of diabetic microangiopathy are observed in the liver of patients with refractory ascites. This suggests a specific role of diabetes in the development of refractory ascites.

P0214

PREDICTORS OF RESPONSE AND OUTCOME TO TERLIPRESSIN IN PATIENTS WITH HEPATORENAL SYNDROME

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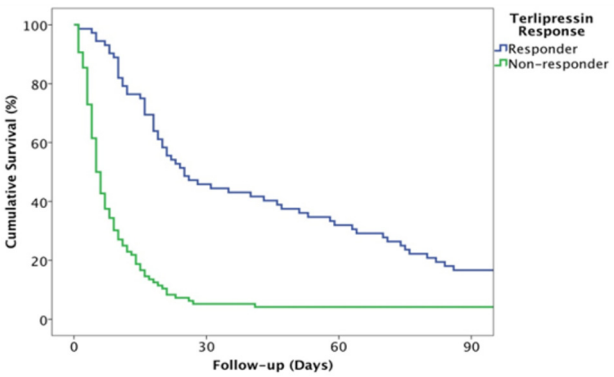
Background and Aims: Renal failure is the most powerful predictor of death in decompensated cirrhosis. We conducted this prospective, observational, cohort study to determine the factors influencing hepatorenal syndrome (HRS) development, survival and determine predictors of response and outcome in terlipressin responders.
Methods: Cirrhotic patients were diagnosed with AKI as per (IAC/ADQI – International Ascites Club/Acute Dialysis Quality Initiative) definition and evaluated as per the study protocol after taking written informed consent and applying inclusion exclusion criteria. The patients who received terlipressin were analysed for predictors of terlipressin response. Those patients with diagnosis of HRS were analysed for the factors influencing its development and survival.
Results: 395 consecutive cirrhotic patients with renal impairment defined as per protocol definitions were included. Out of these AKI patients HRS was seen in 85 patients (21.5%). Terlipressin was given to 162 patients, 72 (44%) patients responded to terlipressin as per the study definition. 90 (56%) patients were terlipressin non responders. HRS had the worst survival outcome, around 20% at 30 days and less than 10% at 90 days. Significantly worst survival was seen in terlipressin non responders at around 10% and less than 10% at 30 days and 90 days respectively as compared to terlipressin responders with 30 day survival around 50% and 90 day survival at around 20% respectively. Higher MELD score, low Serum albumin, presence of SBP and presence of hypotension with inotrope requirement were more significantly associated with HRS/ATN development whereas HRS mortality did not get affected with infection related parameters Longer duration of hospitalization, higher CTP and MELD score, male gender, presence of HE and hypotension with inotropic support requirement correlated significantly with response to terlipressin on univariate analysis. Presence of hypotension requiring inotropic support was the only factor on multivariate analysis to correlate with terlipressin non response.
Conclusions: Development of HRS is associated with significantly reduced survival than other types of AKI in cirrhotics with especially dismal survival in terlipressin non responders. The terlipressin response rate in our cohort was 44%. Development of hypotension with inotropic requirement was the single most important predictor of terlipressin non response.



P0215

METFORMIN REDUCES HYPERAMMONEMIA IN PORTACAVAL SHUNTED RATS INHIBITING GLUTAMINASE ACTIVITY

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Background and Aims: Metformin has been found able to protect cirrhotic patients against hepatic encephalopathy (HE) (Ampuero et al. 2012). K-type glutaminase activity is increased in HE and it has been proposed as the main source of ammonia in liver disease. The gut, kidney and muscle are the key organs implicated

on HE. The aim of this work was to analyze the effect of metformin on glutaminase (GLS1) gene expression and K-type glutaminase enzyme activity in a rat model of HE.
Methods: RT-PCR and glutaminase activity assays were performed in different tissues from 16 male Wistar rats that underwent portacaval shunt (PCS). Eight animals were treated with 30 mg/kg/day of metformin for two weeks. RT-PCRs were made using SensiFAST™ SYBR Lo-ROX One-Step Kit (Bioline, EEUU) in presence of *Gapdh* and *Gls1* primers (QIAGEN, Germany) in an Eco™ Real Time PCR System (illumina®, EEUU). Glutaminase activity was assayed following the colorimetric protocol described by Heini. Plasma ammonia was measured following the glutamate-dehidrogenase enzymatic assay in a COBAS Integra® 700 (ROCHE, Switzerland).
Results: No changes in *Gls1* gene expression levels in gut, kidney and muscles were found associated with metformin use in PCS rats. However, metformin was associated with a significant inhibition of glutaminase activity levels in small intestine (0.277±0.11 mU/μg vs 0.142±0.07 mU/μg); $p < 0.05$, but not in muscle (0.075±0.054 mU/μg vs 0.054±0.006 mU/μg); p = ns, neither kidneys (0.337±0.22 mU/μg vs 0.286±0.22 mU/μg); p = ns. In non-treated PCS rats, plasma ammonia level reached 238.8±166.1 μg/dL and in PCS rats treated with metformin ammonia levels decreased significantly to 110.0±49.1 μg/dL; $p < 0.01$ (Figure 1).
Conclusions: Metformin treatment reduces hyperammonemia in PCS rats modulating glutaminase activity in small intestine but with and inconsistent effect in muscle and kidneys. No changes in gene expression suggests a postranscriptional effect.

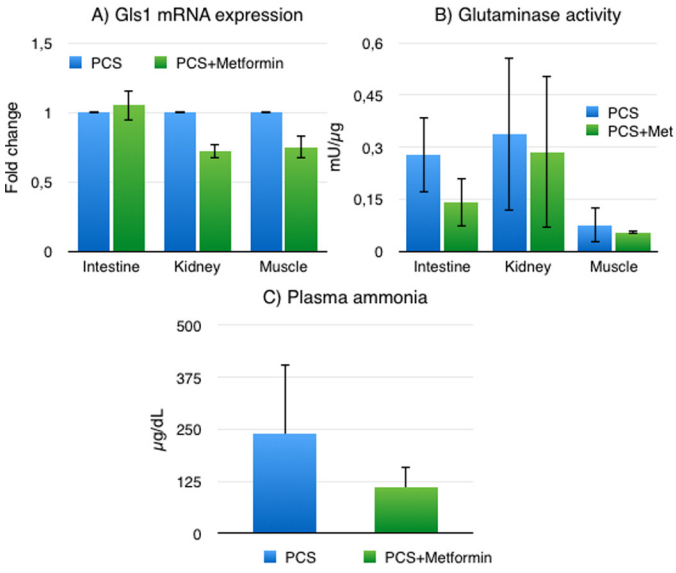

Figure 1. (A) mRNA expression of Gls1 in different tissues after 30 mg/kg/day of metformin treatment. (B) Glutaminase activity results obtained in the same animals. (C) Plasma ammonia.

P0216
INFLUENCE OF PREVIOUS DECOMPENSATION OF CIRRHOSIS ON HYPERDYNAMIC CIRCULATION AND RESPONSE TO β-BLOCKERS IN PRIMARY PROPHYLAXIS OF VARICEAL BLEEDING

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Background and Aims: In cirrhosis with portal hypertension and varices, worsening of liver function increases the risk of variceal bleeding. Hyperdynamic circulation is more developed in decompensated than in compensated cirrhosis, but it is unknown whether it influences the response to β-blockers. The aim of this study was to evaluate whether decompensation of cirrhosis influence the hemodynamic response to β-blockers in primary prophylaxis.
Methods: Patients with cirrhosis and high-risk esophageal varices without previous bleeding, who were referred for primary prophylaxis, were consecutively included in the study. A hepatic and systemic hemodynamic assessment was performed and portal pressure gradient (PPG) was measured before and after i.v. administration of propranolol (0.15 mg / kg). Development of hyperdynamic circulation and response to β-blockers was compared in patients with and in those without previous decompensation of cirrhosis
Results: Among 203 patients included, 114 (56%) had previous decompensation of cirrhosis (ascites in 80%). As compared to patients with compensated cirrhosis, those with previous decompensation had worse Child–Pugh score (5.5±1 vs 7.6±1; $P < 0.001$) as well as each of its components. Alcoholic etiology of cirrhosis was more frequent in patients with decompensation (12% vs 47% $p < 0.001$) while platelet count and rates of splenomegaly and collateral circulation on ultrasound and of gastric varices on endoscopy, were similar in both groups. Patients with decompensation had a more developed hyperdynamic circulation, as indicated by higher cardiac index (4.1±1.3 vs 4.5±1.3 L/min/m^2; P = 0.03) and lower systemic vascular resistance (1042±307 vs 858±282 dyn·s·cm^{-5}; $P < 0.001$). PPG was higher in those decompensated both at baseline (17.5±4 vs 19.3±4 mmHg; P = 0.005) and after β-blocker (P = 0.01). The decrease in GPP achieved with propranolol was lower in patients decompensated, both considering absolute (−2.7±1.8 vs −1.8±2.9 mmHg; P = 0.02) and percentage reduction (−15±9% vs −11±9% mmHg; P = 0.01).
Conclusions: Patients with decompensated cirrhotic and high-risk esophageal varices without previous bleeding have, as compared with those with compensated cirrhosis, in addition to a greater development of hyperdynamic circulation, higher portal pressure gradient and worse acute hemodynamic response to β-blockers

P0217
USE OF BETA-BLOCKERS AT THE ONSET OF SPONTANEOUS BACTERIAL PERITONITIS MAY BE BENEFICIAL DUE TO MODULATION OF IMMUNE RESPONSE

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Background and Aims: After spontaneous bacterial peritonitis (SBP), use of beta-blockers is associated with poor longterm survival. However, animal models suggest a beneficial effect of blocking sympathetic nerve signals during peritonitis. To clarify this issue in humans, we studied the effect of beta-blockers in patients with SBP.
Methods: We determined transplant-free 30-day survival in cirrhotic patients who developed SBP between March 2012 and October 2014 in our department and stratified them for use of beta-blockers. Ascites cells were stimulated with lipopolysaccharide (LPS) for 5 hours after pre-incubation with propanolol. Interleukin 8 (IL-8) was measured with ELISA.
Results: The study population comprised 60 patients with SBP [35 (58%) male, median age 59 years]. Cirrhosis was due to alcohol in 38 (63%) patients, to viral hepatitis in 11 (18%) patients. Seven (12%) patients had hepatocellular carcinoma (HCC). Median MELD score was 21.5, and 46 (77%) patients were classified as Child–Pugh stage C. 37 (62%) and 23 (38%) patients were without

and with beta-blockers at time of SBP, respectively: 14 (61%) with propranolol, the rest with metoprolol, bisoprolol, nebivolol or carvedilol. Clinical and laboratory data were comparable between patients with and without beta-blockers when diagnosis of SBP was made.
30-day survival was 62% (n = 37). Patients on beta-blockers survived longer than patients without beta-blockers (p = 0.033). Poor survival was also associated with the presence of HCC (p = 0.010) and MELD score >22 (p < 0.001). A forward conditional Cox logistic regression analysis confirmed MELD score >22 (p < 0.001) and use of beta-blockers (p = 0.032) as prognostic factors of 30-day survival. Frequency of acute kidney failure during the 30 days after SBP was comparable between patients with (63%) and without (81%) beta-blockers (p = 0.22).
Patiens with beta-blockers had higher fractions of mononuclear cells among the ascites leukocytes (0.26) compared to patients without beta-blockers (0.19; p = 0.029). In addition, IL-8 levels in a subgroup (n = 20) of patients where ascites was available were lower in patients with (513 pg/ml) compared to patients without beta-blockers (1035 pg/ml) (p = n.s.) Pre-treatment with 5 μg/ml propanolol attenuated the increase of IL-8 production when non-infected ascites was stimulated in-vitro with LPS to mimick SBP (p = 0.033).
Conclusions: Use of beta-blockers at the onset of SBP may be beneficial due to modulation of immune response.

P0218

FACTORS ASSOCIATED WITH HEMODYNAMIC RESPONSE TO PROPRANOLOL AND CARVEDILOL IN CIRRHOTIC PATIENTS

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Background and Aims: Sequential measurements of hepatic venous pressure gradient (HVPG) are used to assess the response to non-selective betablockers (NSBBs). We explored the association of different baseline parameters, changes in heart rate (HR), blood pressure during NSBBs, NSBBs doses and HVPG response.
Methods: Our retrospective study included 284 patients with paired HVPG measurements. The association between different baseline demographic characteristics, laboratory parameters, etiology and severity of liver disease, NSBBs doses, baseline HR/blood pressure and changes in HR/blood pressure and achievement of HVPG response was analyzed. Response (R) to NSBB was defined as decrease of HVPG – drop ≥20% or to absolute values <12 mmHg, while partial response (PR) was defined as HVPG decrease ≥10% but <20%, and non-response (NR) as a HVPG decrease <10%.
Results: 133 patients (46.8%) were responders, 47 (16.6%) partial responders, and 104 (36.6%) were non-responders (NR).
For propranolol (154 paired measurements), the following parameters were associated in univariate analysis with achievement of HVPG response: absence of history of variceal bleeding, lower baseline HVPG values, lower GGT, lower Forns score, higher basal HR and pronounced decrease in HR (Table). The following parameters did not influence the achievement of HVPG response to propranolol: age, gender, BMI, etiology of liver disease, HCC presence, presence/history of ascites or hepatic encephalopathy, Child–Pugh class, baseline bilirubin, albumin, AST, ALT, platelet count, MELD score, APRI, FIB-4, Fibrosis Index score, King's, Lok score, propranolol dose, baseline systolic, diastolic or mean blood pressure, decrease in systolic, diastolic or mean blood pressure. In multivariate analysis, decrease in HR (p = 0.0003) and Forns score (p = 0.02) reached the statistical significance.
A decrease in HR >22% had 57.6% Se, 71.8% Sp, 66.6% PPV, 63.6% NPV and 64.9% accuracy for predicting HVPG full response to propranolol (AUC = 0.688), while a Forns score <9.8 had 76% Se, 44.2% Sp, 56.8% PPV, 65.3% NPV and 59.7% accuracy (AUC = 0.623).
For carvedilol (130 paired measurements), only the decrease of systolic blood pressure reached the significance in univariate analysis (Table).
A decrease in systolic blood pressure >12% had 49.1% Se, 74% Sp, 59.5% PPV, 65.1% NPV and 63.1% accuracy for predicting HVPG full response to carvedilol (AUC = 0.629).
Conclusions: None of the baseline characteristics, laboratory tests or changes in HR or blood pressure during NSBB treatment can predict HVPG response to propranolol or carvedilol with sufficient accuracy.

Table (abstract P0218).

Factor	Responders (R)	Partial responders (PR)	Non-responders (NR)	p values
Propranolol				
History of variceal bleeding	n = 30 (39.4%)	n = 8 (42.1%)	n = 35 (59.3%)	R vs PR: 0.96 R vs. NR: **0.03** PR vs. NR: 0.19
Basal HVPG (mmHg)	13.6±3.6	20±4.5	19.1±4.6	R vs PR: **<0.0001** R vs. PR: **<0.0001** PR vs. NR: 0.45
GGT (U/l)	101 (IQR: 61–180)	129 (IQR: 101–309)	153 (IQR: 96–247)	R vs PR: 0.07 R vs. NR: **0.01** PR vs. NR: 0.97
Forns	8.4±2.1	9.3±2.7	9.3±2.1	R vs PR: 0.09 R vs. NR: **0.02** PR vs. NR: 0.31
Basal heart rate (bpm)	80.8±13.6	76.3±14.6	74.7±14.3	R vs PR: 0.20 R vs. NR: **0.01** PR vs. NR: 0.68
Decrease in HR (%)	−24 (IQR: −14.7 to −33.5)	−13.3 (IQR: −3.5 to −21.2)	−15.7 (IQR: +6.6 to −25.6)	R vs PR: **0.0006** R vs. NR: **0.001** PR vs. NR: 0.29
Carvedilol				
Decrease in systolic blood pressure (%)	−10.3 (IQR: −2.7 to −20.3)	−2.5 (IQR: +8.8 to −11.4)	−6.7 (IQR: +3.3 to −13.4)	R vs PR: **0.006** R vs. NR: **0.04** PR vs. NR: 0.12

P0219

PROPHYLACTIC ADMINISTRATION OF BLOOD PRODUCTS DOES NOT PREVENT BLEEDING AFTER ENDOSCOPIC BAND LIGATION OF VARICES

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Background and Aims: Prophylactic administration of platelets and fresh frozen plasma (FFP) is recommended in cirrhotic patients with low platelets/prolonged INR and esophageal varices (EV) that are submitted to endoscopic band ligation (EBL); however it is unknown if this measure prevents post-EBL bleeding. In this analysis we evaluated the outcome of patients undergoing EBL and the role of pre-procedure administration of blood products in this setting.
Methods: Retrospective analysis of consecutive EBL procedures in patients with cirrhosis and EV performed at Hospital Clinic, Barcelona from 2010 to 2013. FFP and platelet transfusion were administered if INR was >1.5 and/or platelet count $<50\times10^9$/L. Complications related to post-EBL bleeding and the relationship with administered blood products were recorded.
Results: 516 EBL procedures were performed in 231 patients (70% male), etiology: HCV and alcohol (77%), median MELD 12, Child A/B/C (40/45/15%). EBL procedures were performed for primary (127, 25%) or secondary (290, 55%) prophylaxis and for acute variceal bleeding (AVB) (99, 20%). Median procedure per patient was 2 (1–3). Bleeding occurred in 25 procedures (4.8%). Bleeding complications were not different between Child A/B vs. Child C patients ($p = 0.5$) and occurred independently of cut-off values and FFP/platelet transfusion (Table 1). FFP and platelets were given in 41 (7.9%) and 39 (7.6%) procedures respectively. Multivariate analysis showed that MELD score (OR, 1.1; 95% CI, 1.0–1.2; $p < 0.01$) and AVB (OR, 3.4; 95% CI, 1.6–7.0;$p < 0.01$) emerged as a predictive factors for post-EVL bleeding.
Conclusions: Pre-procedure administration of FFP and platelets in cirrhotic patients undergoing EBL does not confer a benefit as a prophylactic measure of post-EBL bleeding. Post-EBL bleeding is independent of pre-procedure cut-off values and FFP/platelet administration. MELD score and AVB are risk factors for post-EBL bleeding. Prospective studies are needed to determine the role of routine administration of blood products prior to EBL in patients with cirrhosis.

Table 1. Blood products prior to EBL according to pre-set cut off values

	n	Post-EBL bleeding, n (%)		*p* value
		Yes	No	
INR >1.5				0.17
+ FFP transfusion	41	4 (9.8%)	37 (90.2%)	
no FFP transfusion	48	1 (2.1%)	47 (97.7%)	
Platelets $<50\times10^9$/L				0.57
+ platelet transfusion	39	1 (2.6%)	38 (97.4%)	
no platelet transfusion	29	2 (6.9%)	27 (93.1%)	

Liver tumors: a. Experimental

P0220

JNK SIGNALING ACTIVATED BY PLATELET-DERIVED GROWTH FACTOR D (PDGF-D) STIMULATES SECRETION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-C (VEGF-C) BY CANCER-ASSOCIATED FIBROBLASTS TO PROMOTE LYMPHANGIOGENESIS AND EARLY METASTASIZATION IN CHOLANGIOCARCINOMA

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Background and Aims: Cholangiocarcinoma (CCA) is a highly invasive malignancy with a poor prognosis. Currently, <30% of CCA patients are candidates to surgical resection, due to the early lymph node metastasization. In CCA, lymphangiogenesis develops within an abundant stroma, mainly composed by cancer-associated fibroblasts (CAF) recruited, among others, by PDGF-D secreted by CCA cells. Mechanisms governing lymphangiogenesis in CCA are not known. We aimed to investigate the role of PDGF-D-mediated epithelial–mesenchymal interactions in promoting lymphangiogenesis.
Methods: Immunohistochemical studies of human CCA specimens ($n = 6$), were performed to understand the relationship among lymphatic vessels ($D2\text{–}40^+$), blood vessels ($CD34^+$), CCA cells and CAF (αSMA^+), and the expression of PDGF-D, PDGFRβ, VEGF-C and VEGFR3, as compared with the peritumoural areas. Furthermore, lymphatic microvessel density (LMVD) and vascular microvessel density (VMVD) were calculated in CCA and compared with hepatoma (HCC) ($n = 6$). Secretion of lymphangiogenic growth factors (VEGF-C, Ang-1 and Ang-2) was measured by ELISA in cultures of primary fibroblasts challenged with PDGF-D with/without the PDGFRβ inhibitor, imatinib mesylate (IM), and the JNK inhibitor, SP600126. Using Boyden chambers, we next evaluated the motility of human lymphatic endothelial cells (LEC) following stimulation with conditioned medium of PDGF-D-challenged fibroblasts with/without SP600126.
Results: With respect to HCC, CCA was characterized by a marked increase in LMVD and a reduction in VMVD. In CCA, LEC laid in close vicinity to CAF and to cancer cells. We observed a specific expression of PDGF-D on CCA cells, PDGFRβ and VEGF-C on CAF, and VEGFR-3 on LEC, suggesting a sequential cross talk from tumoral bile ducts to lymphatic vessels via CAF. This was confirmed by *in vitro* results showing that upon PDGF-D stimulation, fibroblasts secreted VEGF-C but not Ang-1 (Ang-2 was not expressed), an effect significantly reduced by IM, and by SP600126. Conditioned medium of PDGF-D-stimulated fibroblasts induced a significantly stronger effect on LEC recruitment than that of unstimulated fibroblasts, and this effect was significantly hampered by JNK inhibition.
Conclusions: PDGF-D released by CCA cells stimulates CAF to secrete VEGF-C by activating JNK signaling. In turn, VEGF-C secreted by CAF stimulates LEC recruitment. This cross-talk identifies PDGFRβ and JNK as targets for antilymphagiogenic therapies in CCA.

P0221

ACQUIRED RESISTANCE TO SORAFENIB IN HEPATOCELLULAR CARCINOMA IS MEDIATED BY TUMOR INITIATING CELLS (CANCER STEM CELLS)

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Background and Aims: Sorafenib is the only systemic therapy approved for the treatment of hepatocellular carcinoma (HCC), but most patients ultimately relapse resulting in disease progression. The precise molecular mechanisms underlying acquired resistance relevant for planning second line therapies are yet unknown. Herein, we explore the role of tumour-initiating cells (T-ICs) – *cancer stem cells* – in the acquisition of resistance to sorafenib and the signalling pathways involved in this process.
Methods: HCC xenograft mice treated with sorafenib (n = 26) were explored for long-term sensitivity (n = 5) and development of acquired resistance (n = 17, median= 42 days). To underscore the mechanism of acquired resistance we assess: (1) the role of T-ICs by in vitro sphere formation assays, and in vivo tumorigenesis assays using NOD/SCID mice, (2) the role of alternative signaling as mediator of resistance and (3) the efficacy of anti-FGF therapy in a mouse model of HCC. Gene expression profiling (microarray, qRT-PCR) and protein analysis (immunohistochemistry) were conducted. Gene pattern modules were used to generate a gene signature of sorafenib resistance and tested in 2 independent cohorts of HCC patients (n = 442).
Results: Acquired resistance to sorafenib developed after a median of 42 days in 65% of mice. There was a significant enrichment of T-ICs in sorafenib-acquired resistant tumours (164 cells needed to create a tumour) vs. sorafenib-sensitive tumours (13400 cells needed) and vs. non-treated tumours (1292 cells needed), $p < 0.001$. Tumours with sorafenib-acquired resistance were enriched with IGF and FGF signaling activation cascades and gene sets of resistance to antitumoral therapies (FDR <0.05). We generated a sorafenib-resistance 175-gene signature that was enriched with progenitor-cell features and predicted poor survival in two independent cohorts of HCC patients (n = 223 and n = 219, respectively), $p < 0.05$. This gene signature was associated with molecular subclasses of poor outcome and aggressive tumours. Furthermore, mice showing acquired resistance treated with an FGF-inhibitor (brivanib) vs. sorafenib showed delayed tumor growth, improved survival, and inhibition of FGFR1 activity and downstream signaling.
Conclusions: Sorafenib-induced acquired resistance is mainly driven by T-ICs with enrichment of progenitor markers and activation of IGF and FGF signaling. Inhibition of these pathways would benefit a subset of patients after sorafenib progression.

P0222

RELEVANCE OF ORGANIC CATION TRANSPORTER OCT1 (SLC22A1) IN DIETHYLNITROSAMINE-INITIATED AND PHENOBARBITAL-PROMOTED HEPATOCELLULAR CARCINOMA IN OCT3-KNOCKOUT MICE

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Background and Aims: We have recently shown a down-regulation of OCT1 (SLC22A1) and OCT3 (SLC22A3) in human hepatocellular carcinoma (HCC) and human cholangiocarcinoma (CCC), associated with tumor progression and a worse patient survival. The OCT1 down-regulation in HCC may affect the ability of sorafenib and platin derivates to reach active intracellular concentrations in these tumors.
Methods: Liver tumors were induced in OCT3-knockout ($OCT3^{-/-}$) mice and wildtype (WT) mice with Diethylnitrosamine (DEN) and Phenobarbital over 10 month. SLC22A1 mRNA expression was measured in tumors and corresponding non-neoplastic tumor surrounding tissue (TST) by real time (RT)-PCR. Protein expression was determined by western blot analysis. Tumor characteristics and blood parameters were assembled. $OCT3^{-/-}$ and WT mice underwent bile duct ligation (BDL) or placebo surgery (Sham operation). After 7 days mice were sacrificed and SLC22A1, TNF-α, TGFβ, IL-10 and col1α1 mRNA expression were measured by RT-PCR in liver tissue. Fibrosis was quantified by Sirius Red staining. Finally mRNA and protein expression of potentially regulatory factors and pathways were investigated by RT-PCR and western blot analysis respectively.
Results: In accordance to the findings in human HCC SLC22A1 mRNA and protein expression were down regulated in liver tumors compared to TST in $OCT3^{-/-}$ mice ($p = 0.001$). Though $OCT3^{-/-}$ mice do not present an obvious phenotype, tumor size ($p < 0.01$) and quantity ($p = 0.001$) were enhanced in comparison to WT mice. Tumors in $OCT3^{-/-}$mice showed significant higher proliferation ($p \leq 0.01$), apoptosis ($p = 0.002$), fibrosis ($p = 0.004$), steatosis ($p < 0.001$) and inflammation ($p = 0.047$). $OCT3^{-/-}$ mice developed significantly more fibrosis 7 days after BDL. OCT1 mRNA expression was up regulated in ligated and Sham operated $OCT3^{-/-}$mice and tended to decrease after BDL. We found increased levels of TNF-α, TGFβ and col1α1 mRNA expression and a decrease in Il-10 mRNA expression in ligated $OCT3^{-/-}$ mice. Finally HNF4α and PPAR-α mRNA expression were down regulated in tumors vs. TST in $OCT3^{-/-}$ mice. Western blot analysis showed significant alterations in Ras-Raf-MEK-ERK, JNK and mTOR signaling pathways.
Conclusions: The down-regulation of OCT1 in liver tumors in $OCT3^{-/-}$ mice is associated with enhanced tumor growth and $OCT3^{-/-}$ mice are more susceptible for liver damage. The expression of OCT1 might be regulated via HNF4α and PPAR-α in $OCT3^{-/-}$ mice and different signaling pathways are involved.

P0223

ROLE OF ARL6IP5 IN HCV-RELATED HEPATOCELLULAR CARCINOMA

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Background and Aims: Recent studies have suggested that ARL6IP5 (ADP-ribosylation-like factor 6 interacting protein 5) may function as a tumour suppressor gene in cancers. Our preliminary studies

have revealed significantly increased expression level of ARL6IP5 in HCV-infected individuals, particularly in HCV-related HCC tissues. The aim of this study was to examine the biological role of ARL6IP5 in the development of liver cancer.
Methods: Real time quantitative PCR (qPCR) and Western blot were used to examine the expression of ARL6IP5 in human hepatocellular carcinoma (HCC) tissues and matched non-cancerous liver. HuH7 cells stably over-expressing ARL6IP5 and cells with stable silencing of ARL6IP5 were used to test for tumorigenesis in nude mice. The effect of ARL6IP5 modulation on cell proliferation, migration and invasion was tested in HCC cells using BrDU incorporation, wound healing, and Boyden Chamber assays, respectively.
Results: Higher levels of ARL6IP5 were found in HCC tissues than in non-HCC tissues. In HCC tumors, those derived from HCV infected individuals had higher levels of ARL6IP5 than those without prior HCV infection. Following acute DEN treatment of mice, there was a transient increase in the hepatic expression of ARL6IP5, which was paralleled with enhanced expression of hepatic IL-6. In DEN-induced mouse HCC, hepatic expression of ARL6IP5 in the tumors was significantly higher than that in adjacent non-cancerous liver. In nude mice studies, xenograft tumors derived from ARL6IP5 overexpressing HuH7 cells showed no difference compared to xenografts derived from control vector expressing cells with regard to tumor volume and growth velocity. In contrast, xenograft tumors derived from HuH7 cells with stable ARL6IP5 knockdown showed significantly reduced tumor growth velocity and tumor volume. *In vitro* studies showed that transfection of HuH7 cells with HCV core JFH1 led to a marked increase in the expression of ARL6IP5 both at the mRNA and protein levels, and the HCV-induced increase of ARL6IP5 was partially reversed by treatment with interferon a2A. Down-regulation of ARL6IP5 in HCC cells by siRNA-ARL6IP5 led to decreased cell proliferation, decreased migration, and reduced cellular invasion.
Conclusions: These findings strongly suggest that ARL6IP5 may be an important mediator for HCV-induced inflammation, and it may play an important oncogenic role in liver cancer formation.

P0224
NINTEDANIB PREVENTS THE DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN A MOUSE MODEL OF FIBROSIS
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Background and Aims: Hepatocellular carcinoma (HCC) affects 30% of cirrhotic patients, which represents 1% of the world population. To date, no molecular therapies have been approved for HCC prevention in these patients. In this study, we aimed to evaluate the chemopreventive efficacy of the tyrosine kinase inhibitor (TKI) nintedanib (VEGFR1–3, FGFR1–3, PDGFRα/β inhibitor; Boehringer Ingelheim) in a mouse model of HCC.
Methods: HCC was induced in the context of fibrosis in C57Bl/6 male mice (n = 55) by a single intraperitoneal (i.p.) injection of DEN (25 mg/kg) at day 15 postpartum followed by weekly i.p. injections of CCl_4 (0.5 ml/kg) from 4 weeks of age. 12 weeks-old mice were then randomized to receive nintedanib (50 mg/kg) (n = 29) or vehicle (n = 26). Mice were sacrificed at 15, 17 and 18 weeks of age. Blood and liver samples were collected. Primary study end-points included HCC incidence, number and size of macroscopic tumours. Molecular analysis was assessed at gene expression (microarray) and protein level (western blot and immunohistochemistry). Drug tolerance was evaluated by body weight loss and plasma AST/ALT levels.
Results: Nintedanib was well tolerated and reduced HCC incidence in treated mice compared to control group at 15 weeks (13% vs 43%), 17 weeks (30% vs 89%, $p < 0.02$) and 18 weeks (90% vs 100%). The number of tumours/mouse significantly decreased at 17 weeks (0.9 vs 4.7, $p = 0.003$) and 18 weeks (3.5 vs 11.4, $p = 0.003$). Nintedanib also prevented the development of large number of tumours (>4 tumours) at 17 weeks ($p = 0.01$) and 18 weeks ($p = 0.008$). Tumour size was significantly reduced at 17 weeks (0.7 mm vs 6.4 mm, $p = 0.01$) and 18 weeks (4.8 mm vs 16.2 mm, $p = 0.001$). Gene expression profiling showed 471 deregulated genes (Fold change >1.5, $p < 0.05$) between the control and treated group. In addition, IPA and GSEA revealed the capacity of nintedanib to reverse the pro-carcinogenic phenotype of control mice (inflammation, tissue damage response, epithelial–mesenchymal transition, stem cell pluripotency) and to promote liver function in treated mice. Furthermore, nintedanib blocked the activation of VEGFR2 and the downstream effectors AKT and ERK. Histological analysis confirmed the development of HCC in the context of fibrosis.
Conclusions: Nintedanib is able to prevent the development of HCC in a fibrosis-based experimental model. These data provide the rationale for testing the therapeutic potential of TKIs in cirrhotic patients at high-risk to develop HCC.

P0225
COMBINATION OF CD40L CO-STIMULATION WITH ALPHA-FETOPROTEIN PULSED DENDRITIC CELLS INDUCED AN EARLY AND STRONG TH1-SHIFT IN THE TUMOR ENVIRONMENT AND SYNERGISTIC ANTITUMORAL EFFECTS IN SUBCUTANEOUS AND ORTHOTOPIC MURINE HEPATOCELLULAR CARCINOMA MODEL
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Background and Aims: Dendritic cells (DC) as professional antigen presenting cells can be used to prime T-cells against the tumor-associated antigen α-fetoprotein (AFP) for immunotherapy of hepatocellular carcinoma (HCC). However, a strong immunosuppressive tumor environment limits their efficacy in patients. The co-stimulation with CD40Ligand (CD40L) is critical in the maturation of DC and T-cell priming. In this study, the impact of CD40L to improve vaccination with murine (m)AFP-pulsed DC (Ad-mAFP-DC) was analyzed in poor immunogenic subcutaneous (s.c.) and orthotopic murine HCC.
Methods: Murine DC cultured with GM-CSF and IL-4 were adenovirally transduced with Ad-mAFP, Ad-CD40L or Ad-LacZ (control). Hepa129-mAFP-cells were injected into the right flank or the liver of C3H-mice to induce subcutaneous (s.c.) and orthotopic HCC. For treatments, 10^6 mAFP-pulsed DC were inoculated s.c. followed by 10^6 CD40L-expressing DC (Ad-CD40L-DC) injected intratumorally (i.t.).
Results: Ad-mAFP-DC, which were co-cultured with Ad-CD40L-DC showed a CD40L-dependent enhanced immunostimulatory capacity. S.c. inoculation with this mixed vaccine induced complete protection towards challenge with mAFP-positive HCC cells in more than 30% of animals compared to controls. In pre-

established tumors, intratumoral (i.t.) application of Ad-CD40L-DC combined with s.c. inoculation with Ad-mAFP-DC also enhanced the antitumoral effects, achieving complete remissions and long-term survival in 62% of animals. Analysis of the tumor environment at different time points revealed that s.c.-vaccination with Ad-mAFP-DC stimulates tumor-specific effector cells, allowing an earlier Th1 shift and recruitment of effector T-cells within the tumors. After i.t. co-stimulation with Ad-CD40L-DC, production of Th1-cytokines was strongly increased and accompanied by a robust tumor infiltration of mature DC, activated $CD4^+$-, $CD8^+$-T-cells as well as reduction of regulatory T-cells. Moreover, Ad-CD40L-DC induced tumor cell apoptosis.

Conclusions: Co-stimulation with CD40L significantly improves vaccination with Ad-mAFP-DC in a protective setting and in pre-established HCC *in vivo*. Combined therapy causes an early and strong Th1-shift in the tumor environment as well as higher tumor apoptosis, leading to effective and synergistic tumor regression of HCC. Thus, CD40L co-stimulation represents a promising tool for improving DC-based immunotherapies of HCC.

P0226

REDUCTION IN SUMOYLATION-DEPENDENT S100A4 NUCLEAR IMPORT IN CHOLANGIOCARCINOMA BY LOW DOSE PACLITAXEL HALTS TUMOR INVASIVENESS AND HEMATOGENOUS METASTATIZATION BY MODULATING RHO-A AND CDC42 ACTIVITIES

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Background and Aims: Cholangiocarcinoma (CCA) is characterized by early and strong invasiveness. Nuclear expression of the S100A4 protein is a marker of increased CCA invasiveness. We aimed at studying if S100A4 nuclear expression promotes CAA invasiveness and may be a druggable therapeutic target. Preliminary experiments showed that Paclitaxel (PTX), a microtubule stabilizing agent, is able to reduce the nuclear import of S100A4.

Methods: CCA cells expressing nuclear S100A4 (EGI-1) were treated with increasing PTX doses to study *in vitro* effects on nuclear and cytosolic S100A4 expression, nuclear S100A4 sumoylation (a process regulating nuclear import of several proteins), cytoskeletal integrity, cell proliferation, apoptosis, motility, invasiveness and Rho GTPases activity.

To confirm *in vitro* data, SCID mice were xenografted with EGI-1 by spleen injection. After confirming tumor engraftment by bioluminescence imaging, we treated mice with PTX at a low-dose metronomic regimen (2.6 mg/kg/die; n = 5) for 14 days; untreated mice served as controls (n = 5). We then evaluated PTX *in vivo* effects on primary tumor size, number of micrometastasis (MM) and isolated tumour cells (ITC) in liver and lung tissue samples, along with proliferation, apoptosis and S100A4 expression of EGI-1.

Results: PTX at 1.5 and 15nM induced a marked reduction in nuclear S100A4 without modifying its cytoplasmic levels. The decrease of nuclear S100A4 was linked with a significantly reduced sumoylated fraction and a relevant attenuation of both cell migration and invasiveness, without affecting proliferation/apoptosis or cytoskeletal integrity. Furthermore, Rho-A and Cdc42 activities were reduced. In xenografted mice, PTX induced a reduction in both the primary tumor size and number of lung MM and ITC. In the primary CCA mass of PTX-treated mice, the number of EGI-1 expressing nuclear S100A4, were significantly reduced, whereas proliferation/apoptosis rate did not change.

Conclusions: Down-regulation of nuclear S100A4 by PTX at doses well below those commonly used in chemotherapy regimens but able to interfere with the sumoylation process, results in a decrease of CCA cell motility and invasiveness in conjunction with a reduction of hematogenous spread. These effects do not depend on changes in cell proliferation, apoptosis or cytoskeleton integrity, but on the reduction of Rho-A and Cdc42 activities caused by reduced S100A4 nuclear translocation. This is a novel therapeutic target in CCA.

P0228

OVER-EXPRESSION OF SURVIVIN ENHANCES THE CHEMOTHERAPEUTIC EFFECTIVENESS OF YM155 IN HUMAN HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide and displays inherent resistance to most of the chemotherapeutic drugs. Survivin (BIRC5) belongs to the family of inhibitor-of-apoptosis proteins (IAPs) which antagonizes the induction of cell death. Dysregulated expression of IAPs is frequently observed in cancers, and the comparative high levels of survivin in tumors make it an attractive target for pharmacological interventions. The small imidazolium-based compound YM155, a "survivin suppressant", has been employed to block the expression of survivin via inhibition of the survivin promoter. Although YM155 has been shown to be well tolerated, but unfortunately it's clinical efficacy has been insofar only moderate. Here, we have studied the molecular mechanism(s) that regulate the expression of survivin in HCC cells and to perform preclinical efficacy evaluation of the survivin inhibitor, YM155, as a therapeutic agent against HCC.

Methods: We have studied the quantitative expression of survivin in human HCC biopsies and a panel consisting of 25 HCC cancer cell lines. The orthotopic mouse xenograft model was employed to monitor the therapeutic effects of YM155 in vivo.

Results: We demonstrated the remarkable heterogeneity of survivin protein expression in human HCC biopsies and cell lines. High survivin expression in HCC patients' samples correlated with comparatively poorer survival. HCC cancer cell lines also exhibited a difference in sensitivity to YM155 with the high survivin expresssor cell lines were significantly more sensitive than the low expressor cells to YM155. The sensitivity of HCC cell lines to YM155 was associated with the increase expression of phosphorylated survivin. YM155 was found to inhibit cell growth and induced cell cycle arrest by altering the gene expressions of genes associated with DNA damage, cell cycle, and apoptosis in human HCC cells. Importantly, YM155 yielded significantly better therapeutic effects than sorafenib when tested in an orthotopic xenograft HCC model using HCC patient-derived xenografts with high survivin expression.

Conclusions: Biomarker-driven oncology trials represent a paradigm shift in drug discovery and the promise of precision medicine. Our results provide the scientific basis for designing actionable, targeted therapy of employing YM155 to treat HCC patients whose tumor express elevated level of phosphorylated survivin in "adaptive" clinical studies.

P0229

JAGGED2-MEDIATED NOTCH SIGNALING REGULATES CELLULAR PLASTICITY AND PROMOTES A CANCER STEM CELL PHENOTYPE IN HCC

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Background and Aims: Cancer cells within tumors can exist in various states of differentiation and can flux between these states, a phenomenon mediated by epigenetic changes. Determining epigenetic changes is critical for effective therapy because dedifferentiation can drive a cancer stem cell (CSC) phenotype with more aggressive behaviour that is resistant to current therapies. Notch signalling is a major mediator of cell fate and is a critical signalling pathway in maintaining the cells in an undifferentiated state. Our study characterises the role Notch signalling plays in maintaining a LCSC phenotype.
Methods: Undifferentiated cells with a liver cancer stem cell (LCSC) phenotype were isolated from HCC cell lines HuH7 and PLC/PRF/5 using flow cytometry based on the expression of the classical CSC marker Oct4. The expression of Notch signalling components were examined in these undifferentiated cells by Western blot. Tumour sphere assays and cell proliferation were undertaken on undifferentiated cells treated with recombinant human Jagged2 (rhJagged2) and siRNA specifically targeting Jagged2 (siRNA-Jagged2). The expression of transcription factors involved in dedifferentiation was then determined in the HCC cell lines. The expression of Jagged2 in tumor and adjacent non-tumor tissue from HCC patients was evaluated by Western blot.
Results: Increased expression of Jagged2 and the Notch functional receptor subunit NICD1 was observed in Oct4+ LCSCs. Treatment of LCSCs with rhJagged2 resulted in activation of Notch signalling as manifested by increased expression of Notch signalling components, particularly the Notch downstream targets Hey1 and NICD1. On the other hand, inhibition of Jagged2 by siRNA led to a significant reduction in the expression of Notch signalling components. rhJagged2-treated LCSCs showed increased cell proliferation and tumor sphere formation which were significantly blunted by siRNA-Jagged2. HCC cells PLC/PRF/5 and HuH7 treated with rhJagged2 resulted in a significant up-regulation of Sox-2. Increased expression of Jagged2 was also observed in human HCC tumour tissues compared to adjacent non-tumour liver.
Conclusions: Jagged2 is likely an important molecule in the maintenance of CSC phenotype and differentiation status; this in turn is involved in HCC development. Up-regulation of Jagged2 in HCC tissues suggests that Jagged2 targeting may be a therapeutic option for HCC. Studies are currently underway to explore the therapeutic benefit of targeting Jagged2 in HCC.

P0230

STARD1 OVEREXPRESSION EXACERBATES CHOLESTEROL-MEDIATED HEPATOCARCINOGENESIS

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Background and Aims: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide and a complication of advanced liver disease. Cholesterol is an emerging factor promoting fatty liver disease. Moreover, StARD1, a protein responsible for cholesterol trafficking to mitochondria, has been found to have a role in steatohepatitis and HCC. Although recent findings showed that obesity and hepatic steatosis promotes tumorigenesis, the role of cholesterol in HCC progression has not been elucidated yet. Thus, the aim of this study was to investigate the role of cholesterol and StARD1 in chemically-induced hepatocarcinogenesis and the therapeutic effect of ezetimibe.
Methods: 14 days-old mice were injected i.p. with a single dose of diethylnitrosamine (DEN). 30 days later, mice were fed with high fat diet (60% calories from fat) containing cholesterol (0.5%) (HFCD) supplemented or not with ezetimibe (10 mg/kg/day) for 32 weeks. Overexpression of hepatic Stard1 was induced by genetically-engineered adenovirus in mice treated with DEN and fed the HFCD. Liver samples were processed to detect tumor markers by Western Blotting, IHC and qRT-PCR.
Results: Liver cholesterol was significantly higher in the HFCD fed group compared to HFCD+ezetimibe or regular diet group. The expression of the proliferation markers Ki-67 and PCNA induced by DEN was elevated in HFCD fed mice and this effect was reduced in mice treated with ezetimibe. In addition, DEN-induced fibrosis, measured by Sirius red staining, hydroxyproline, collagen1A1 and osteopontin expression was further exacerbated in mice fed the HFCD, and ezetimibe reversed this increase. DEN-induced expression of tumor markers CK-19, AFP and GP-73 was elevated in the HFCD group compared to HFCD+ezetimibe. The multiplicity (average of tumors/mice) and tumor burden (average weight of tumors/mice) observed after 8 months were significantly higher in mice fed the HFCD compared with the HFCD+ezetimibe group. Moreover, 100% of mice died in HFCD group vs 50% in HFCD+ezetimibe group after 1 year. Additionally, StARD1 overexpression induced by adenovirus, doubled the multiplicity of tumors and induced a parallel increase in expression of tumor markers when compared to animals injected with control empty adenovirus.
Conclusions: High cholesterol in liver promotes the growth and progression of HCC and ezetimibe reverses these effects. StARD1 overexpression increases HCC, likely by stimulating the trafficking of mitochondrial cholesterol.

P0231

EFFECT OF PERIFOSINE ALONE AND IN COMBINATION WITH SORAFENIB IN AN HrasG12V PLUS shp53 TRANSGENIC MOUSE MODEL OF HEPATOCELLULAR CARCINOMA GENERATED BY HYDRODYNAMIC INJECTION

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Background and Aims: Novel therapeutic strategies are needed to improve survival in patients with hepatocellular carcinoma (HCC). Perifosine has shown antitumor activity by inhibition of Akt phosphorylation in many advanced solid tumors. This study was to investigate the effect of perifosine alone and in combination with sorafenib in a HrasG12V plus short-hairpin RNA downregulating p53 (shp53) double transgenic mouse model of HCC.
Methods: The mouse model of HCC was generated by hydrodynamic injection of transgenes HrasG12V/shp53, and the mice were treated with perifosine with or without sorafenib to evaluate effects of drugs on tumor growth and survival. Tumor cell proliferation and tumor angiogenesis were evaluated by immunohistochemical analysis of Ki-67 and CD31, respectively. Tumor cell apoptosis was detected by using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay. Levels of key enzymes in the PI3K/Akt, Ras/Raf/MAPK, and caspase pathways were detetected by western blot analysis, and levels of vascular endothelial growth factor (VEGF) were determined by immunohistochemistry and western blot analysis.

Results: Treatment with perifosine for 5 weeks, alone and in combination with sorafenib, strongly inhibited tumor growth and increased survival. Perifosine inhibited HCC cell proliferation, induced apoptosis, and decreased tumor angiogenesis. Furthermore, its combination with sorafenib enhanced these effects. In addition, Akt phosphorylation was decreased by perifosine and further decreased by combination treatment. Although perifosine alone did not appear to activate the caspase pathway, combination treatment increased the cleavage of caspase-3, caspase-9, and poly (ADP-ribose) polymerase. Perifosine did not affect VEGF levels, as assessed by immunohistochemistry and western blot analysis.
Conclusions: Results of this in vivo study indicate that perifosine may be an effective drug for the treatment of HCC. Furthermore, their application in combination with sorafenib may have significant advantages compared with single-drug treatment in the treatment of HCC.

P0232
REDUCED TRANSCRIPTIONAL RESPONSIVENESS TOWARDS THE ANTI-CANCER DRUG IRINOTECAN IS MEDIATED BY A COMMON UGT1A HAPLOTYPE
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Background and Aims: A standard neoadjuvant therapy of colorectal cancer includes treatment with irinotecan. Its active metabolite SN-38 undergoes glucuronidation by different UDP-glucuronosyltransferases (UGT). A combination of several SNPs in the *UGT1A* genes (UGT1A1*28, UGT1A7*3) is associated with a higher risk for irinotecan toxicity including late diarrhea and leucopenia. To prevent unanticipated preoperative hepatotoxicity imposed by neoadjuvant chemotherapy, liver function including glucuronidation activity should represent an important consideration in therapeutic strategies. Aim of this study was therefore the analysis of irinotecan-mediated *UGT1A* gene regulation in presence and absence of *UGT1A* SNPs, as well as the expression of common *UGT1A* activating transcription factors (TF) [Aryl hydrocarbon receptor (AhR), NF-E2-related factor 2 (Nrf2)].
Methods: Humanized transgenic (htg)*UGT1A-WT* and *-SNP* (containing 10 common *UGT1A* SNPs) mice were treated with 25 mg/kg irinotecan for 4 days. RNA was isolated and mRNA expression was determined by TaqMan-PCR.
Results: In *htgUGT1A-WT* mice, irinotecan treatment led to the transcriptional upregulation of UGT1A1, UGT1A3, UGT1A4 and UGT1A9 mRNA in the liver (1.3–6 fold) and in the jejunum (2–10 fold). However, the highest irinotecan induced *UGT1A* upregulation was detected in the colon of *htgUGT1A-WT* mice (4–17 fold). In contrast, absolute *UGT1A* expression levels were reduced in *htgUGT1A-SNP* mice compared to *htgUGT1A-WT* mice. Analysis of the expression of TFs revealed that AhR expression was mainly induced in the liver, whereas Nrf2 expression was increased in the liver and in the intestine.
Conclusions: This is the first demonstration of *UGT1A* regulation by irinotecan. In contrast to the common assumption that the liver represents the most important organ for SN-38 glucuronidation, the considerable intestinal upregulation of *UGT1A* expression by irinotecan indicates that the intestine may be a significant location of SN-38 glucuronidation. Upregulation of AhR and Nrf2 by irinotecan indicates their direct role in *UGT1A* activation by irinotecan. The presence of *UGT1A* SNPs was associated with reduced *UGT1A* expression levels after irinotecan treatment in comparison to *htgUGT1A-WT* mice, which is a likely explanation for a higher risk for side effects in patients with *UGT1A* polymorphisms. Our data implicate that this effect should be considered as a risk factor for neoadjuvant therapies in patients with metastatic colorectal cancer and anticipated hepatic surgery.

P0233
MICRORNA-210 ENHANCES TUMOR ANGIOGENESIS BY TARGETING FGFRL1 IN HEPATOCELLULAR CARCINOMA
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Background and Aims: Hypoxia is implicated in many aspects of tumor development and progression, especially for solid tumors like hepatocellular carcinoma (HCC). The master hypoxiamir miR-210 is upregulated in HCC tumor tissues, but its pathophysiological role and the relevance of the up-regulation to HCC has not been well established.
Methods: We analyzed miR-210 expression by qPCR in normal liver tissues, adjacent tumor tissues and hepatocellular carcinoma.
Results: We found that the expression level of miR-210 increases gradually from normal liver tissues to adjacent tumor tissues and to incipient and advanced tumor tissues. In HCC patients, high expression of miR-210 in tumor tissues associates with poor prognosis, on both overall survival ($P < 0.05$) and tumor free survival ($P < 0.05$). HCC tumors with high levels of miR-210 expression showed high scores of microvascular density by CD34 immunostaining. The pro-angiogenic role of miR-210 confirmed and evaluated through both *in vitro* and *in vivo* studies. Fibroblast growth factor receptor like 1 (FGFRL1) is a known direct target of miR-210. We demonstrate that extracellularly secreted FGFRL1 could attenuate the pro-angiogenic effects of fibroblast growth factor 2. By reducing FGFRL1, miR-210 might participate to cope with hypoxia stress during tumor genesis and development in HCC.
Conclusions: Our findings uncover a novel mechanism of hepatocarcinogenesis through enhancing angiogenesis by up-regulation of miR-210 in HCCs, which is correlated with poor prognosis, and open the perspective of targeting FGFRL1 for HCC drug development.

P0234
A TGF-BETA GENOMIC SIGNATURE IN INTRAHEPATIC CHOLANGIOCARCINOMA HIGHLIGHTS A NOVEL LONG NON CODING RNA LOCATED IN THE NUCLEAR PARASPECKLES OF TUMOR CHOLANGIOCYTES
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Background and Aims: Intrahepatic cholangiocarcinoma (ICC) is the second most common type of primary cancer in the liver after hepatocellular carcinoma (HCC). ICC is an aggressive malignancy with a poor prognosis and limited therapeutic options. Recently, we reported that the overexpression of TGF-beta in the stroma of ICC correlates with a poor prognosis, suggesting that TGF-beta contributes to ICC progression. The aim of the present study is to investigate the role of TGF-beta and associated target genes, including long non coding RNA (lncRNA), in ICC.
Methods: Experiments were conducted in HuCCT1 and Huh28 ICC cell lines stimulated by TGF-beta. Gene expression profiling was done using pangenomic microarrays. The expression of relevant target genes was validated by RT-PCR. Cellular localization of lncRNA was determined by in situ hybridization in human ICC using tissue microarrays. Functional analysis was done by gain and loss of function experiments using expression plasmids and shRNA.
Results: We show that Huh28 cells display a high expression of mesenchymal markers (e.g. SNAI1, VIM) while epithelial markers (e.g. EPCAM, CDH1) are highly expressed in HuCCT1. In agreement with a mesenchymal phenotype, Huh28 cells exhibit a constitutive

activation of the TGF-beta pathway. Next, we established TGF-beta associated gene signatures in the two cell lines. Statistical analysis identifies 859 genes differentially expressed by TGF-beta ($p < 0.001$) in HuCCT1 and 491 genes in Huh28 cells, among those about 300 are in common. Interestingly, the signature includes lncRNA similarly deregulated in HuCCT1 and Huh28. The deregulation of known and new TGF-beta target genes is validated, including T-LINC-1, a lncRNA strongly up-regulated by TGF-beta. T-LINC-1 is induced by TGF-beta in ICC, HCC and hepatic stellate cells. TGF-beta inhibitors decrease the expression of T-LINC1 in Huh28 cells. Profiling of microdissected ICC shows that T-LINC-1 is up-regulated in tumor cholangiocytes and stromal cells. In situ hybridization reveals that T-LINC-1 localizes in the paraspeckles. Paraspeckles are subnuclear bodies critical to control of gene expression and acts as retention centers for specific RNA. Gain and loss of function experiments demonstrate that T-LINC-1 modulates the expression of NEAT1, another lncRNA required for paraspeckle structure.
Conclusions: Our study identifies a novel lncRNA induced by TGF-beta which may play a critical role in carcinogenesis by modulating paraspeckle activity in tumor cells.

P0235
PROTEIN N-TERMINAL ACETYLATION INHIBITION AS A NOVEL THERAPEUTIC TARGET FOR HEPATOCELLULAR CARCINOMA
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Background and Aims: The identification of new targets for systemic therapy in the treatment of hepatocellular carcinoma (HCC) is one of the main needs in HCC treatment. We have recently observed that hNatB enzyme catalyses N-*terminal acetylation* of 10% human proteome, being necessary for a proper activity of actin cytoskeleton, organelle with relevant implications in tumour development. Therefore, the aim of this study was to explore the therapeutic potential of NatB inhibition in HCC and its implication in HCC development and HCC patient prognosis.
Methods: hNatB expression was inhibited in HCC human cell lines, Huh7 and PLC/PRF/5, and the effects on cellular proliferation, signalling pathways and tumoral development were analysed in xenotransplant mouse model. Finally, hNatB subunits expression was analysed in paired tumor and non-tumoral samples from 31 HCC patients in BCLC A (74%) and B (26%) stages treated with liver resection or transplantation. The association of NATB expression and clinical, radiological and histological variables related to HCC prognosis was analysed. Chi-squared test was used for statistical analysis.
Results: hNatB expression inhibition resulted in the blockade of Huh7 and PLC/PRF/5 cell lines proliferation and in the impairment of their tumoral properties. This effect was associated with actin cytoskeleton disorganisation that results in YAP oncogene inactivation and in a reduced Epidermal Growth Factor (EGF)-mediated ERK activation. An overexpression of hNatB in tumor compared to non-tumor samples was observed in more than half of HCC patients (51%). Moreover, the overexpression of both NatB subunits was associated with microscopic vascular invasion ($p = 0.04$). No association between NatB overexpression and tumor stage, multinodularity or tumor differentiation was observed.
Conclusions: hNatB mediated protein N-terminal acetylation could be a new therapeutic target for the treatment of HCC and its overexpression could be related to more aggressive phenotype of HCC as it is associated with microvascular invasion.

P0236
ROLE OF YAP SIGNALLING DEREGULATION IN THE GENETIC SUSCEPTIBILITY TO HEPATOCARCINOGENESIS, AND STEMNESS AND AGGRESSIVITY OF LIVER CANCER
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Background and Aims: Recent observations suggest a contribution of YAP upregulation to hepatocellular carcinoma progression (HCC). We analyzed the connection of YAP deregulation with genetic susceptibility to hepatocarcinogenesis and HCC stemness and aggressivity.
Methods: HCC was induced in F344 and BN rats, genetically susceptible and resistant to hepatocarcinogenesis, respectively. Human HCCs with poorer prognosis (<3 years survival, after partial liver resection, HCCP) and HCCs with better outcome (>3 years survival; HCCB) were archival samples. Gene expression was evaluated by quantitative PCR and immunoblotting, and functional experiments were done with HepG2, Huh7, and Hep3B liver cell lines.
Results: Higher upregulation of Yap and of its target Ctgf, in F344 rat HCC than in BN HCC, was associated with highest increase in Yap-tyr357, p73 phosphorylation, and Caspase 3 cleavage in BN HCC. Upregulation of YAP, CTGF, 14–3-3 and YAP-14–3-3 complex, TEAD and YAP-TEAD complex in HCC reached highest values in HCCP. In contrast, YAP-ser127 decreased in HCC with lowest values in HCCP, and YAP-tyr357, p73 phosphorylation and Caspase 3 cleavage showed highest increase in HCCB. Stem cell markers NANOG, OCT3–4, and CD133 upregulation progressively increased from HCCB to HCCP and was significantly correlated to YAP and YAP-TEAD expression. Growth rate was 2.5–3 times lower, between 48 and 96 hours, in HepG2 s than in Huh7 and Hep3B cells. This was associated, at 48 hours, with lower YAP and NANOG, OCT3–4, and CD133 expression in HepG2 than in Huh7 and Hep3B cells. YAP downregulation by specific siRNA in Huh7 and HepG3 cells led to significant decrease in NANOG, OCT3–4, and CD133 expression. In contrast, sharp upregulation of stem cell markers was induced in HepG2 cells by forced *YAP* overexpression.
Conclusions: Our results indicate: (1) YAP signalling deregulation in HCC is under genetic control. (2) Genetic resistance to HCC is associated with highest phosphorylation of Yap at tyr357 and p73, and highest apoptosis. (3) YAP changes favoring the formation of YAP-14–3-3 and YAP-TEAD complexes, associated with cell survival, contribute to HCC aggressivity. In contrast, YAP changes favoring apoptosis, such as phosphorylation at tyr357, are associated with better HCC prognosis. (4) YAP upregulation favors HCC stemness and aggressivity.

P0237
HEPATOCELLULAR CARCINOMA: APPLIED GENOMICS TO DIAGNOSTIC AND PERSONALIZED THERAPY
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Background and Aims: The application of targeted therapies as a result of precise molecular diagnosis of human cancer is now improving the clinical outcome of patients with melanoma (BRAF), breast (HER2) or lung cancer (EML-ALK) but not of those with hepatocellular carcinoma (HCC). From a genomic perspective, HCC is very heterogeneous with a low mutational recurrence and in advanced stages, sorafenib offers a suboptimal efficacy (OS =

11 months). We have decided to analyze specific mutational signatures to support precise molecular diagnostics with potential to guide the design of individualized targeted therapies.
Methods: *Selection of HepatoExoma:* Based on studying the NGS data already available for HCC, we have designed a targeted NGS platform (HepatoExoma) to study, in individual lesions, the somatic mutations detected among a selection of "actionable" genes potentially associated with inhibitory drugs. This can allow the characterization of specific microclonal dynamics and potentially guide the use of targeted therapies based on specific mutational signatures. For this, we have focused on studying the coding DNA of 114 genes using an enrichment library kit (HaloPlex, Agilent) compatible with ultrasequencing approaches.
Samples: (A) Commercial HCC cells with a known mutational profile (CCLE-Broad Institute). (B) Paired genomic DNA (normal vs tumoral) from HCC patient samples obtained from transplantation, resection or biopsy.
Results: *In silico:* We have confronted the genes included in our design to the WES data from 88 patients (Xiao Liu, 2013). We could detect alterations in 67% of the patients and up to 45.5% of these, with 2 "actionable" mutations that we can potentially associate to a targeted treatment using 2 drugs alone or in combination. HCC cell lines showed and average of 11 mutations with HepG2 (i.e.) being susceptible to targeted treatment using 4 drugs.
Experimental: (A) Biological and functional studies of specific targeted therapies for HCC cells. (B) Mutational data of 9 patients analyzed by HepatoExoma.
Conclusions: (1) The HepatoExoma allows the generation of data that can potentially guide a personalized therapy in 45.5% of HCC patients.
(2) Mutational data from preclinical HCC models allowed us to explore the effects of rationally designed targeted therapies with improved activity.
(3) In the near future, we will be able to test these therapies in murine xenografts or isolated hepatocyte from patients.

P0239
TUMORIGENIC POTENTIAL OF CANCER STEM CELLS (CSCS) ISOLATED FROM HUMAN CHOLANGIOCARCINOMA (CCA) SUBTYPES IN CIRRHOTIC MICROENVIRONMENT

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Background and Aims: Cholangiocarcinomas (CCA) are comprised histologically of a mucin-secreting type, which can be intrahepatic (IHCCA) or perihilar (pCCA), and, of a mixed type located peripherally within the liver. We characterized cancer stem cells (CSCs) in CCA subtypes and evaluated their cancerogenic potential.
Methods: CSC markers (CD90, CD13, EpCAM, CD133, LGR5) were investigated in 25 human CCAs, in primary cultures and established cell lines. Tumorigenic potential was evaluated in vitro or in xenografted mice after subcutaneous or intrahepatic injection in normal and cirrhotic (CCL4) mice.
Results: CSCs comprise more than 30% of the tumor mass. While the CSC profile was similar between mucin-IHCCA and mucin-pCCA, CD13+ CSCs characterize the mixed-IHCCA while LGR5+ characterize the mucin-CCA subtypes. A significant percentage of neoplastic cells express epithelial–mesenchymal transition (EMT) markers (SNAIL, Twist), and co-express mesenchymal and epithelial markers. In primary cultures, EMT markers, mesenchymal markers (vimentin, CD90), and CD13 were largely more expressed than the "epithelial" markers (CD133, EpCAM and LGR5). In vitro, CSCs expressing "epithelial" markers formed a higher number of spheroids than CD13+ or CD90+ CSCs. In subcutaneous tumor xenografts, tumors dominated by stromal markers were formed primarily by CD90+ and CD13+ cells. By contrast, in intrahepatic xenografts, in the cirrhotic livers, tumors were dominated by epithelial traits resembling the original human CCAs.
Conclusions: CSCs were rich in human CCAs (>30%), implicating CCAs as stem cell based diseases. Different CSC subpopulations expressed EMT markers and co-expressed mesenchymal and epithelial markers, generating different types of cancers depending on the microenvironment. Remarkably, CSCs may reproduce the original human CCAs, when injected into cirrhotic livers.

P0240
PROTEOMIC ANALYSIS OF TISSUE INTERSTITIAL FLUID FOR IDENTIFICATION OF NOVEL SERUM CANDIDATE DIAGNOSTIC MARKER FOR HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma (HCC) is the fifth most common malignant cancer in the world. The sensitivity of alpha-fetoprotein is still inadequate for clinical diagnosis of HCC and identification of new biomarkers is greatly needed.
Methods: Tissue interstitial fluid (TIF), as the liquid microenvironment of cancer cells, has been demonstrated as a promising material for biomarker discovery. In order to identify proteins highly expressed in HCC TIF for further validation, a proteomic technique named iTRAQ (isobaric tag for relative and absolute quantitation) was used to analyze paired tumor and nontumor TIF samples from 6 HBV-HCC patients.
Results: Totally, 241 up-regulated proteins (ratio tumor/nontumor ≥1.3, $p < 0.05$) and 288 down-regulated proteins in tumor TIF ($ratio_{tumor/nontumor} \leq -1.3$, $p < 0.05$) were identified. Proteins involved in lipid metabolism, cell cycle, cellular movement and cell morphology were up-regulated in HCC samples, while proteins involved in amino acid metabolism were down-regulated. Interestingly, we found the up-regulation of S100 family proteins and down-regulation of glutathion-s-transferase and leptin related proteins in tumor. One dramatically up-regulated protein S100A9 ($ratio_{tumor/nontumor}=19$) which plays a prominent role in the regulation of inflammatory processes and immune response was further validated by ELISA in sera from liver cirrhosis (LC, HCC high risk population) and HCC patients ($n = 47$ for each group). The level of this protein was significantly elevated in HCC sera (256 ± 167 ng/ml) compared with LC (112 ± 119 ng/ml). The area under the curve of this protein to distinguish HCC from LC was 0.83, with sensitivity of 91% and specificity of 66%.
Conclusions: Our result till now showed that S100A9 could be a candidate HCC biomarker for further validation in larger populations. And TIF was a promising material to identify candidate tumor biomarkers that could be detected in serum.

P0241

CD40/CD40LIGAND INTERACTION BETWEEN TUMOR LYSATE-PULSED DENDRITIC CELLS INDUCES A TH1-ENVIRONMENT AND ENHANCES THE CYTOTOXIC ACTIVITY OF IMMUNOLOGIC EFFECTOR CELLS TOWARDS HUMAN HEPATOCELLULAR CARCINOMA AND CHOLANGIOCELLULAR CARCINOMA CELLS

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Background and Aims: Dendritic cells (DC) are professional antigen presenting cells able to prime T-cells against tumor-associated antigens (TAA). The interaction of CD40 on DC and CD40L on effector cells is a potent co-stimulatory signal for an effective cellular immune response. In this study, the impact of CD40/CD40L-interactions between human DC on the induction of tumor-specific effector cells was analyzed towards human hepatocellular (HCC) and cholangiocellular (CCC) carcinoma cells *in vitro*.
Methods: Adenoviral vectors encoding human (h)CD40L were generated using the Adeno-Easy system. Peripheral blood lymphocytes were isolated from healthy donors by Ficoll density gradient-centrifugation to obtain DC and lymphocytes. DC were pulsed with tumor-lysates and transduced with Ad-hCD40L or Ad-Mock (control) on day 6. Expression of DC-maturation marker and cytokines were studied by flow cytometry and ELISA respectively. Lymphocytes were stimulated with CD3, IL-2, IFN-gamma and IL-1beta and co-cultured with DC. Ability to lyse HCC- (HepG2, Huh7 and CCL13) or CCC-cells (EGI-1 and Mz-ChA-2) was determined using a LDH (lactate-dehydrogenase)-based cytotoxicity assay. Apoptosis of tumor cells was studied by quantification of sub-G1-fraction by flow cytometry.
Results: Transduction with Ad-hCD40L induced a strong interaction between human DC due to the expression of hCD40L in more than 35% of DC. CD40/CD40L between the human DC induced a strong expression of Th1 cytokines, such as IL-12 and IFNgamma in DC-supernatant, as well as increased expression of CD83 (90%) and CCR7 (60%) on DC surface compared to Ad-Mock-transduced DC or non-transduced DC ($p < 0.05$). Moreover, specific lysis of human HCC- or CCC-cells was significantly ($p < 0.05$) higher, when effector cells were co-cultured with Ad-hCD40L-transduced DC than Ad-Mock-transduced or non-transduced DC. Interestingly, coculturing of tumor cells with the supernatant of hCD40L-expressing DC was able to induce a significant higher apoptosis in tumor cells.
Conclusions: Adenoviral-mediated expression of hCD40L is able to induce a strong CD40/CD40L-interaction between human DC. This interaction induces a Th1-environment increasing significantly the stimulatory capacity of TAA-pulsed DC and the apoptosis induction towards HCC- and CCC-cells. Thus, these results underline the potential of CD40/CD40L-interaction to improve the immunotherapy of TAA-pulsed DC towards HCC and CCC.

P0242

LIVER FIBROSIS PROMOTES HEPATOCARCINOMA GROWTH ACCORDING TO THE SEVERITY OF FIBROSIS IN AN ORTHOTOPIC TUMOR MODEL IN MOUSE

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Background and Aims: HCC is associated in 90% of cases with cirrhosis. However, the fibrosis-dependent mechanisms of hepatocarcinogenesis remain poorly understood. MMP-2, -9 and Membrane-Type 1 (MT1)-MMP are endopeptidases that cleave the extracellular matrix and are related to HCC invasiveness. Our aim was to study the impact of fibrosis and its severity on HCC development and to evaluate the tumor expression of MMP-2, -9 and MT1-MMP according to the liver fibrosis status in an orthotopic transplantation model.
Methods: The HCC cell line Hepa 1–6 is syngenic with the C57Bl/6 mouse strain and is characterized by a constitutive expression of αFP. Hepa 1–6 cells (1×10^6) were injected into the liver by direct puncture in 3 groups of mice ($n = 10$/group): in non-fibrotic liver (normal liver group-NLG), in mild fibrotic liver (mild fibrosis group-MFG) and in severe fibrotic liver (severe fibrosis group-SFG). Mild and severe fibrosis were induced by CCl_4 injections for 2 and 7 weeks respectively. Mice were sacrificed 2 weeks post HCC cell injection. The liver was sliced and examined for the presence of tumor (nodule ≥1 mm). The tumor volume (TV) and the liver to body weight ratio (LW/BW) were used as parameters of tumor burden. A part of each tumor was used for histological analysis and the other for RNA preparation.
Results: A tumor nodule was observed in 60%, 80% and 100% of animals in the NLG, MFG and SFG respectively. The TV and the LW/BW were significantly higher in the MFG ($p = 0.05$ and $p = 0.04$) and in the SFG ($p = 0.03$ and $p = 0.02$) compared to the NLG. Both parameters were also higher in the SFG compared to the MFG ($p = 0.03$; $p = 0.04$). Compared to surrounding liver, αFP mRNA expression was high in all tumors, and significantly higher in SFG tumors compared to both MFG and NLG tumors ($p = 0.02$ and $p = 0.005$, respectively). Intra-tumor expression of MMP-2, -9 and MT1-MMP gradually and significantly increased with fibrosis severity at the time of tumor implantation (NLG < MFG < SFG). Furthermore, there was a positive and significant correlation between TV and LW/BW and the intra-tumor expression of all MMPs.
Conclusions: Our results demonstrate that liver fibrosis promotes HCC development in our model and suggest a link between the severity of fibrosis and the ability of liver cancer to develop. Moreover, we have shown an enhanced MMP-2, -9 and MT1-MMP expression in the tumors of the fibrosis group suggesting a role of the MMPs and matrix remodeling in the fibrosis-dependent promotion of HCC development.

P0243

ROLE OF S100A8/A9 IN CARCINOGEN-INDUCED HEPATOCELLULAR CARCINOMA ONSET AND DEVELOPMENT

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Background and Aims: S100A8 and S100A9 are calcium binding proteins which play a prominent role in the regulation of inflammatory processes. They predominantly form functional anti-parallel heterodimers (S100A8/A9), termed calprotectin. In the extracellular space they act as danger signals by bindung to different receptors such as RAGE (Receptor for Advanced Glycation-End products). Epidemiological studies show increased expression of S100A8 and S100A9 in human hepatocellular carcinoma (HCC), and a strong correlation of both proteins with poorly differentiated cancer. *In vitro* and *in vivo* results suggest a role of calprotectin in liver inflammation and damage, and a pro-tumorigenic function in HCC.
We already demonstrated that ablation of *Rage* impaired tumor development in the inflammation-driven *Mdr2*$^{-/-}$ model, where it reduced number and size of tumors and limited liver damage. On the contrary, in the cirrhosis-free diethylnitrosamine (DEN)

mouse model *Rage* depletion did not influence HCC development, emphasizing that RAGE is essential only in settings of chronic injury.
Absence of *S100a9* in the inflammation-driven *Mdr2*$^{-/-}$ model did not affect HCC development nor liver damage. To address the question if a different tumor microenvironment can influence the role played by S100A8/A9 in HCC onset and development, we investigated hepatocarcinogenesis in an inflammation-free context.
Methods: *S100a9*$^{-/-}$ mice were treated with the procarcinogen DEN, an alkylating agent that causes initial liver damage and promotes DNA-strand breaks, thus leading to HCC formation. Mice were scrificed at 6, 9 and 12 month of age in order to characterize the livers at premalignant and malignant stages.
Results: Lack of *S100a9* significantly reduced the size of tumors in the DEN model. This reduction in tumor size was associated with reduced tumor cell proliferation. Additionally, *S100a9*$^{-/-}$ mice exhibited a higher number of apoptotic cells.
Conclusions: In the *Mdr2*$^{-/-}$ model, the absence of an obvious phenotype due to *S100a9* ablation suggests that other molecules compensate for the lack of calprotectin in tumor development. Our results demonstrate that in contrast to HCC development preceded by chronic hepatitis, liver damage and fibrosis, S100A8/A9 affects hepatocarcinogenesis in an inflammation-free context, where compensatory mechanisms might be absent.

P0244
THIENO-TRIAZOLODIAZEPINES BLOCK HEPATOCARCINOMA PROGRESSION BY HSP-DEPENDENT KINOMA INHIBITION

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Background and Aims: Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. So far there is no effective chemotherapeutic treatment for HCC and the prognosis of advance stage remains poor. The thieno-triazolodiazepine are well known anti-inflammatory drugs that act as PAF-receptor antagonists. Recently an antineoplastic pleiotropic effect have been shown in hematologic tumors where treatment with two different thieno-triazolodiazepine WEB2086 and WEB2170 induces differentiation, growth arrest and apoptosis in leukemia cells. The effects of these drugs on solid tumors such as HCC remain untested.
Aim of this study was to investigate the anti-tumor efficacy of the thieno-triazolodiazepines in in vitro and in vivo models of HCC.
Methods: We tested the effect of WEBs treatment in several hepatoma cell lines evaluating proliferation, apoptosis, invasiveness, cell cycle, autophagy and senescence. We also studied the in vivo efficacy of these molecules in a HBV transgenic mouse model, that spontaneously develop hepatic tumors, and in nude mice injected subcutaneously with human HCC cells.
Results: WEBs were able to reduce the proliferation of cancer cells as assessed by thymidine incorporation and to eliminate the ability of these cells to form colonies in soft agar. This abrogation of anchorage dependent and independent cell growth was associated with cell cycle arrest, apoptosis, vitality reduction, and senescence induction. In addition, WEBs impair hepatoma cell migration and chemoinvasion. WEBs administration in HBV transgenic mice reduce the number and the dimension of tumors, and abrogated the levels of alpha-fetoprotein. Antitumorigenic effects in vivo were also confirmed in a xenograft model of HCC. A 2D-DIGE proteome study highlights that the WEB2086 inhibits the binding processes in particular inhibits protein binding of ATP and GTP and abrogation of this cellular function results kinoma inhibition. In addition 2D-DIGE analysis showed that WEBs down-regulate chaperone proteins such as hsp90 and hsp70 that are involved in protein refolding, an important mechanism in tumor cel resistence. The down-regulation of Hsp90 leads to failure of "client" protein refolding and mediates their degradation.
Conclusions: The thieno-triazolodiazepine are able to reduce HCC progression in human and murine HCC models blocking cancer cell proliferation and migration, and inducing apoptosis and senescence probably whit a mechanism that involve kinome inhibition.

P0245
THE IL-6/SIL6R AND STAT3 AXIS IS CRUCIAL FOR HEPATOPROTECTION AND SUPPRESSION OF LIVER CANCER IN FEMALE MICE WITH CHRONIC LIVER INJURY

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Background and Aims: Hepatocelluar carcinoma (HCC) commonly develops on a background of chronic hepatitis. Evidence from experimental animal models based on chemical-induced HCC associated with *acute* liver injury, suggests that the inflammatory cytokine, interleukin-6 (IL-6), is a crucial, gender-determined factor driving hepatocarcinogenesis. However, in *chronic* liver injury models, IL-6 is also known to be hepatoprotective. As such, its role in chronic hepatitis-associated liver cancer remains unresolved. We investigated the role of IL-6 and signal transducer and activator of transcription 3 (Stat3), one of the main mediators of IL-6 signal transduction, in liver cancer in multidrug resistant gene 2 (*Abcb4*) knockout mice (Mdr2$^{-/-}$), a physiologically relevant model of chronic hepatitis-associated HCC.
Methods: IL6 knockout mice (IL-6$^{-/-}$), and hepatocyte-targeted Stat3 knockout mice (Stat3$^{\Delta hep}$), were crossed into Mdr2$^{-/-}$ mice generating Mdr2$^{-/-}$IL6$^{-/-}$ and Mdr2$^{-/-}$Stat3$^{\Delta hep}$ mice, respectively. Additionally, a transgene encoding sgp130Fc – a selective inhibitor of IL-6 *trans*-signaling mediated by the soluble IL-6 receptor (sIL-6R) – was introduced into Mdr2$^{-/-}$ mice to generate Mdr2$^{-/-}$sgp130Fc mice. Female littermates were followed over the course of 16 months for analysis of hepatitis and tumor development.
Results: Mdr2$^{-/-}$IL6$^{-/-}$ developed increased liver injury and fibrosis, accompanied by reduced Stat3 phosphorylation, in comparison to Mdr2$^{-/-}$ controls. Ultimately, IL-6 deficiency strongly exacerbated tumor development in Mdr2$^{-/-}$ mice. Increased tumorigenesis correlated with a more pronounced inflammatory response, including increased neutrophil and macrophage infiltration to periductal regions, and notably increased dysplastic nodules. Likewise, Mdr2$^{-/-}$sgp130Fc mice displayed a similar phenotype of reduced Stat3 phosphorylation and increased liver injury. Mdr2$^{-/-}$sgp130Fc mice also eventually displayed significantly increased tumorigenesis associated with elevated levels of dysplastic nodules, but without a corresponding change in hepatic inflammation or fibrosis. Finally, hepatocyte-targeted Stat3 inactivation in the Mdr2$^{-/-}$Stat3$^{\Delta Hep}$ mice also resulted in significantly increased liver injury, more and larger dysplastic nodules in the liver parenchyma, and significantly increased tumorigenesis.
Conclusions: Our findings reveal that IL-6 signaling, mediated by sIL-6R and Stat3, promotes resistance to chronic hepatitis and liver cancer in female Mdr2$^{-/-}$ mice.

P0246

POTENT AKT/PKB INHIBITOR REDUCES AKT PHOSPHORYLATION AND TUMOR CELL GROWTH IN VITRO AND IN VIVO

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Background and Aims: The serine/threonine kinase AKT/PKB has a critical role in regulating cell growth by mediating signals from multiple growth factor receptors to a plethora of downstream targets that regulate cell survival, proliferation and apoptosis. Since AKT signaling is deregulated in a number of human malignancies it is an attractive anticancer drug target. Constitutively activating mutations in growth factor receptor genes are a common event in human cancers and result in elevated levels of AKT phosphorylation. Abnormal activation of the AKT pathway is a common event in cancers, most notably in liver, prostate, breast and colorectal carcinoma. Consequently, inhibiting/reducing AKT activity is considered to be an effective approach in anticancer therapy.
Methods: Protein complementation assay based on Renilla luciferase fragments was used to screen small molecule inhibitors from NCI Diversity Set. Reduction of AKT activity and effect on the AKT downstream targets was detected with Western Blot method. Inhibition of cell growth in vivo was confirmed by tumor xenograft model. Tumor xenografts were analysed by immunofluorescence.
Results: We have found a small molecule compound that could be a potent and selective inhibitor of Akt1 signaling pathway in tumor cells. Selected compound reduces Akt activity, inhibits the activity of AKT target proteins that are involved in regulating cell survival, proliferation and metabolism, reduces the growth of tumor cells in vitro and in vivo by increasing the differentiation status of cancer cells. Additional studies are required to elucidate the exact mechanism of Akt activity inhibition. The ability of the compound to reduce growth of human tumor xenografts in nude mice suggests its potential use as a anti-tumor agent and warrants for further research in this direction.
Conclusions: In conclusion, we have identified a potent inhibitor of AKT1-PDPK1 interaction that reduced the level of activated AKT in different experimental settings and shows a high potential to be used as a lead compound for desgning novel anti-tumor agents.

P0247

C1GALT1 IS OVEREXPRESSED IN CHOLANGIOCARCINOMA AND C1GALT1 KNOCKDOWN INHIBITS MALIGNANT BEHAVIORS OF CHOLANGIOCARCINOMA CELLS

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Background and Aims: Cholangiocarcinoma (CCA), arising primarily from the epithelial lining of bile ducts, is a highly lethal liver cancer. O-glycosylation is a common post translational modification of proteins. Beta-1,3-galactosyltransferase (C1GALT1) transfers galactose (Gal) to GalNAc-Ser/Thr (Tn antigen) to form Gal-GalNAc-Ser/Thr structure (T antigen). Tn and T antigens are often associated with tumor progression and altered expression of C1GALT1 has been found to regulate cancer behaviors. However, the expression and function of C1GALT1 in CCA has never been reported. The aim of this study is to investigate the expression of C1GALT1 in primary CCA tissues and the functional roles of C1GALT1 in CCA cells.
Methods: To analyze the expression of C1GALT1, immunohistochemical staining of C1GALT1 in tissue microarrays comprising CCA and normal bile ducts was performed. Knockdown of C1GALT1 with specific siRNAs was performed in CCA cell lines HUCCT1 and SNU1079. Cell viability was analyzed by MTT assay. Cell migration and invasion were analyzed by transwell migration and matrigel invasion assay, respectively.
Results: Our study shows that over 80% of CCA tissues exhibit significantly higher C1GALT1 expression levels than normal bile duct. Moreover, C1GALT1 knockdown significantly suppressed viability, migration, and invasion of CCA cells.
Conclusions: C1GALT1 is frequently up-regulated in CCA and the expression of C1GALT1 is able to regulate the malignant behaviors of CCA cells, suggesting that C1GALT1 is a potential target of therapeutic drug development.

P0248

H-1 PARVOVIRUS: A NEW THERAPEUTIC STRATEGY TARGETING HEPATOCELLULAR CARCINOMA

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Background and Aims: Despite a diverse therapeutic arsenal, survival after treatment of hepatocellular carcinoma (HCC) remains mediocre. These discouraging results make it necessary and urgent to explore new therapeutic strategies. In this sense, the parvovirus H-1 (H-1 PV) is a small oncolytic virus that is capable of selectively destroying cancer cell lines. In this context, the aim of my project is to assess and validate the oncolytic and oncotropic properties of H-1 PV in HCC models to consider its use as an alternative to conventional treatments.
Methods: The oncolytic effect of H-1 PV was evaluated in vitro on a hepatocarcinoma cell line (HuH7) and ex vivo on human HCC explants from hepatectomy pieces and put into primary culture. The cells were infected with increasing amounts of H-1 PV (MOI 1.10 and 100). Cell viability was assessed by a qualitative (microscopy) and quantitative method (MTT). The expression of the viral marker NS1 was analyzed (Western blot) to validate specific H-1 PV lysis. The ability of tumor cells to produce the neo-virions was measured by viral titration (TCID50). These same experiments were used to test the susceptibility of hepatocytes, biliary cells and healthy intrahepatic fibroblasts regarding H-1 PV. Finally, the expression of genes encoding α-SMA and collagen I, III and IV were measured (RT-QPCR) in infected intrahepatic fibroblasts to assess the impact of the virus on the liver fibrogenesis.
Results: The results obtained show that HuH7 as well as tumor hepatocytes in primary cultures are susceptible to H-1 PV-induced oncolysis, with a decrease in cell viability that is dose-dependent and related to the expression of the viral protein NS1. Unlike HuH7, tumor hepatocytes do not produce neo-virions in the culture supernatants. Furthermore, no significant decrease in viability was observed in hepatocytes nor in intratumoral fibroblasts isolated from healthy livers. And, a similar result was obtained for the healthy biliary cells. In these three non-tumor cell types, no expression of the NS1 protein, and no neo-virions were detected. Finally, the analysis by RT-QPCR healthy intrahepatic fibroblasts infected with H-1 PV, shows a decrease in the expression of genes encoding proteins involved in hepatic fibrogenesis.
Conclusions: These experiments show for the first time that H-1 PV is capable of selectively destroying cells from human HCC while sparing healthy cells surrounding the tumor, suggesting new perspectives for HCC treatment.

P0250

EFFECT OF IRREVERSIBLE ELECTROPORATION (IRE) ON THE PORCINE LIVER: COMPARISON WITH RADIOFREQUENCY ABLATION

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Background and Aims: Irreversible electroporation (IRE) is a promising, non-thermal ablation therapy for hepatocellular carcinoma, which, unlike radiofrequency thermal ablation (RFA), induces apoptosis through irreversible formation of nanoscale pores in the cell membrane with delivery of high-voltage electric current and is considered to improve safety in the vicinity of heat-sensitive blood vessel and bile duct. The aim of the present study is to clarify the acute pathological effect of IRE on the liver and also to compare with effect of RFA.
Methods: Three pigs underwent ultrasound-guided IRE and RFA of normal liver under general anesthesia and at laparotomy. A total of 9 IRE ablated areas and 3 RFA ablated areas were created. We examined histopathologic findings of ablated areas at 4 hours after therapy with immunohistochemical methods. The pathologic findings according to IRE ablation conditions were compared with findings of RFA.
Results: In IRE zones, various degree of congestion and hemorrhage, apoptotic change of hepatocytes, defluxion of portal endothelial cells and cholangitis were observed in hepatic lobules. With the increase in the total pulse number of IRE delivered, these pathological findings became remarkable. NADPH-diaphorase enzyme histochemistry performed in IRE zones demonstrated the stainability is very weak in the hepatic lobule, which reflecting defluxion of hepatocytes and reduced activity of viable hepatocytes in acute phase, while the stainability is lost in the central portion of RFA. Silver impregnation stain and collagen type 4 immunohistochemistry showed that reticular fibers and collagen fibers around the sinusoid remain well-preserved in IRE zones, on the other hand, those in RFA zones are very poorly stained. The vessels in IRE zones demonstrated a relatively intact configuration of positive smooth muscle cells for Caldesmon staining and endothelial cells for Factor 8 staining, with the minimal endothelial damage, while the vessels in RFA zones demonstrated a negative or weakened reaction. Bile duct epithelial cells showed a positive reaction for cytokeratin 7 staining in IRE zones with desquamated cells, while an entirely negative reaction in RFA zones, revealing protein denaturation.
Conclusions: Unlike RFA, IRE induces apoptosis of hepatocytes, endothelium of the blood vessels and mucosal membrane of the bile ducts, whereas preservation of sinusoidal framework and vascular structure may have the potential for liver regeneration.

P0251

HBx SENSITIZE HEPATOCELLULAR CARCINOMA CELLS TO LAPATINIB BY UP-REGULATING ErbB3

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Background and Aims: Compelling evidence indicates epidermal growth factor receptor (EGFR/ErbB) family is critical for the tumor progression of hepatocellular carcinoma (HCC) cells, but EGFR/HER2 tyrosine kinase inhibitors (TKIs) showed less therapeutic benefits in unselected HCC patients in clinical trials. Notably, the activations of ErbB family and their downstream signaling pathways have been reported to be associated particularly with the poor prognosis of hepatitis B virus (HBV)-associated HCC, suggesting that HBV may modulate ErbB family signaling. HBV X (HBx) protein has been reported to regulate the cellular sensitivity to chemotherapy, but its roles in therapeutic efficacy of EGFR/HER2 TKIs remain unknown. In this study, we aim to investigate whether and how HBx affects the cellular sensitivity of HCC cells to EGFR/HER2 TKIs.
Methods: The viabilities of HCC cells and their HBx-overexpressing derivatives were analyzed by using MTT, cell counting, and colonogenic assay. The mRNA and protein expression of ErbB family were examined by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) and Western blot analysis. The promoter activity of ErbB3 was determined by luciferase assay.
Results: We unexpectedly found that HBx overexpression renders HCC cell lines more sensitive rather than resistant to lapatinib but not sorafenib. We next investigated the protein and mRNA level of ErbB family and its downstream signaling pathway in HBx expressing cells and their parental cells. We found that ErbB3 was upregulated in HBx expressing cells. Furthermore, luciferase assay indicated that HBx enhanced ErbB3 expression through inducing ErbB3 promoter activity. Enhancing of ErbB3 expression not only sensitized HCC cell lines to Lapatinib but also switch oncogenic addition to ErbB3 signaling pathway. After silencing ErbB3 expression, the cell viability was decreased in HBx-overexpressing cells, further confirming that ErbB3 plays a critical role in HBx-expressing cells. Taken together, our data indicated that HBx and ErbB3 overexpression might be the biomarkers for predicting therapeutic efficacy of lapatinib treatment in HCC patients.
Conclusions: HBx overexpression enhanced the oncogenic addiction of HCC cells to the ErbB2/ErbB3/Akt signaling pathway, and thereby sensitizes HCC cells to lapatinib.

P0252

IDENTIFICATION OF THE APICAL SODIUM BILE SALT TRANSPORTER (ASBT) AS A NOVEL MOLECULAR TARGET TO ENHANCE THE SENSITIVITY OF CHOLANGIOCARCINOMA TO CHEMOTHERAPY

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Background and Aims: The low sensitivity of cholangiocarcinoma (CCA) to available chemotherapy justifies the need of novel chemosensitizating strategies, for instance by enhancing drug uptake, which may be reduced in these tumors. Thus, the expression/function of the organic cation transporter OCT1, involved in the uptake of sorafenib, is markedly impaired in CCA. Bile acid derivatives had been previously investigated to target antitumor agents toward hepatocellular carcinoma. Decreased expression/function of bile acid uptake carriers, such as NTCP, in tumor cells may limit the usefulness of this possibility. The aim of this study was to revisit this pharmacological strategy for CCA, based on preliminary data indicating the expression the bile acid transporter ASBT in these tumors.
Methods: The ursodeoxycholic acid and cisplatin conjugate Bamet-UD2, which is transported by both OCT1 and ASBT, and has antiproliferative activity on tumor cells, but no toxic side-effect in rodents, was used as targeted drug in preclinical in vitro and in vivo models of CCA. Tumors were induced in rats by long-term (30 wk) thioacetamide administration.
Results: The expression by transfection or lentiviral infection of either ASBT or OCT1 enhanced the sensitivity of CCA cells to the anti-proliferative effect of Bamet-UD2, but not cisplatin. The

expression of both transporters in CCA was determined by RT-QPCR. The results confirmed a marked decrease of OCT1 expression in human and rat CCA. Interestingly, ASBT expression was preserved in both human and rat CCA. Moreover, human (TFK1) and rat (NT-92, REUSAL-C44, REUSAL-C49) CCA cells have a negligible expression of OCT1/Oct1 together with, although at different levels, maintained expression of ASBT/Asbt. In these cells, as well as in rat CCA in vivo, efficient uptake of bile acid was suggested by their ability to accumulate taurocholate and the fluorescent bile acid derivative CGamF. In nude mice implanted (s.c.) with tumors expressing ASBT, tumor growth was significantly inhibited by treatment with Bamet-UD2. As compared with cisplatin, the administration (i.p.) of Bamet-UD2 to rats with CCA resulted in an efficient liver uptake with lower exposure of extrahepatic tissues to the drug, which accounted for no sign of toxicity.

Conclusions: In CCA, the expression of OCT1 is markedly decreased as compared with healthy cholangiocytes, whereas that of ASBT is maintained, which suggests that this transporter may be useful for the targeting of cytostatic bile acid derivatives to CCA.

P0253

INHIBITION OF GLYCOLYSIS BY USING NANOLIPID BROMOPYRUVIC CHITOSAN CARRIER IS A PROMISING TOOL TO PREVENT HCC INVASIVENESS

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Background and Aims: An important issue in nanotechnology is that the passage in solid tumour cells will be limited for carriers which have smaller diameter than the range of cancerous cells junctions (100–600 nm). Thus, there is an urgent need to develop smart and efficient strategies tailoring size, shape, and specifically targeting to block one of glycolysis stage. In this way, folic acid (FA) as specific ligand for foliate receptor of cancer cells was conjugated with chitosan (CHI) which was attached with bovine serum albumin (BSA) as target of liver cells forming together (FA-CHI-BSA) to give rise to a specific carrier system. Our aim was to fabricate nano lipid carriers labelled by Rhodamine and covered by two layers: chitosan–bromopyruvic acid complex as one layer then conjugated by BSA-CHI-Folic acid as second layer.

Methods: Charge potential of lipid droplets and intensity of size distribution before and after sonication were investigated by photon correlation spectroscopy. Attachment of Bromopyruvic acid with chitosan was detected by infrared spectroscopy. Conjugation of folic acid and bovine serum albumin (BSA) with chitosan was also detected. Characterization of covering lipid droplets with chitosan–bromopyruvic acid was investigated by SEM, TEM and AFM before and after attachment. The effect of nano-lipid chitosan-BSA-FA encapsulated by bromopyruvic acid on the HCC growth was investigated upon time by crystal violet stain and MTT assay.

Results: Bromopyruvic acid was attached electrostatically by chitosan through amino groups. Molecular fingerprint of bromopyruvic acid, chitosan and complex was detected. Nano-lipid droplets labeled by Rhodamine were covered by chitosan-bromopyruvic acid complex under rotation for 5 min. Afterwards folic BSA-CHI-FA complex was added by completing time of rotation up to 15min. Fluorescence microscopy TRITC and FITC channels revealed successful labelling of lipid droplets covered by complex. Morphological change of HCC growth was shown clearly by crystal violet stain. MTT assay indicates that bromopyruvic acid encapsulated has strongly affected HCC growth. Tryplan blue shows significant increase of apoptotic cells number upon time.

Conclusions: Bromopyruvic acid has exhibited strong effect on HCC growth by blocking hexokinase II enzymes causing mitochondrial potential stress and reduction of ATP energy. Encapsulation of bromopyruvic acid in FA-BSA targeted nano-vehicle might offer protection of normal cell.

P0254

CCR5 EXPRESSED ACTIVATED BUT NOT RESTING REGULATORY T CELLS ACCUMULATED IN THE TUMOR SITE AND CORRELATED WITH STAGES OF HEPATOCELLULAR CARCINOMA

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Background and Aims: Activated regulatory T cells (aTreg) could facilitate the progression of cancer had been demonstrated recently. However, the role of this activated Treg cells and possible underlying mechanisms had not been explored in patients with hepatoma.

Methods: Peripheral blood mononuclear cell (PBMC) from 54 patients with hepatoma and 21 health subjects were obtained. The immunophenotype and functional assays of Treg cells were measured by flow cytometry.

Results: The percentages of aTreg but not resting regulatory T cells significantly increased in peripheral blood and accumulated in the tumor parts in patients with hepatocellular carcinoma. These aTreg had higher expressions of Tumor necrosis factor-α receptor II, cytotoxic T-lymphocyte antigen-4, Ki67 and HLA-DR but very few effector cytokines like IL-2, IL-17A and IFN-g. In addition, an increased expression of CCR5 but not CCR4 on aTreg cells was found in the tumor sites when compared with peripheral blood, indicating CCR5 expressed aTreg cells would preferentially accumulated in the tumor microenvironment. Furthermore, the expression of CCL5, a chemokine for CCR5, increased significantly in tumor tissues as well. Finally, the frequencies of both aTreg and CCR5 expressed aTregs were well correlated with tumor stages of patients with hepatocellular carcinoma.

Conclusions: Activated but not naïve Treg cells increased peripherally and accumulated to the tumor microenvironment in patients with hepatoma. Furthermore, CCL5/CCR5 interaction might be the possible mechanisms for the migration of these aTreg cells into tumor site and be the possible target for the tumor immunotherapy.

P0255

DISRUPTION OF THE GP130 SIGNALING PATHWAY IN HEPATOCYTES ATTENUATES HEPATOCARCINOGENESIS

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Background and Aims: Activation of gp130/IL-6/STAT3 signaling often correlates with tumorigenesis and poor prognosis in hepatocellular carcinoma (HCC) patients. A major disadvantage of global IL-6 anti-cancer therapy is the systemic elevations of IL-6, and new treatment options are needed. Thus, in the present study we aimed to investigate the role of gp130 in hepatocytes for the initiation and progression of HCC.

Methods: Female and male hepatocyte-specific gp130 knockout mice ($gp130^{\Delta hepa}$) and control animals ($gp130^{f/f}$) were treated with diethylnitrosamine (DEN). The role of gp130 for acute injury (0–144 h), tumor initiation (24 weeks) and progression (40 weeks) was analyzed.

Results: After acute DEN-induced injury, a reduction in periportal necrosis, dysplastic foci and in the inflammatory response as

reflected by decreased levels of IL-6, IL-11 and oncostatin M (OSM) were observed in gp130$^{\Delta hepa}$ mice. These results were supported by FACS analysis which showed decreased infiltration of polymorphonuclear leukocytes (PMN) and infiltrating monocytes. Loss of gp130 slightly attenuated HCC initiation 24 weeks after DEN treatment as evidenced by a trend towards less microscopic tumor nodules in gp130$^{\Delta hepa}$ compared to WT livers. In contrast, 40 weeks after DEN treatment, gp130$^{\Delta hepa}$ female and male mice displayed significantly smaller tumors and reduced tumor burden associated with decreased oxidative stress and DNA damage, indicating a role for hepatocyte-specific gp130 expression during HCC progression. Interestingly, while phosphorylation of STAT3 and Ser727 STAT3 was absent in gp130$^{\Delta hepa}$ livers, we found STAT5 activation and decreased pSMAD2/3, SMAD2 and TGFβ-dependent activity.
Conclusions: Our results suggest that gp130 deletion in hepatocytes plays a crucial role in DEN-induced HCC progression. Disruption of gp130 signaling resulted in STAT3 inhibition, increased STAT5 activation and diminished TGFb-dependent signaling. Hence, blocking gp130 in hepatocytes might be a therapeutic approach to inhibit progression of HCC.

P0256
INTERLEUKIN-8 LEVEL AS PREDICTIVE MARKER FOR TREATMENT RESPONSE AFTER TRANSARTERIAL CHEMOEMBOLIZATION IN HEPATOCELLULAR CARCINOMA

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Background and Aims: Interleukin-8 (IL-8) is a multifunctional pro-inflammatory cytokine. Increased IL-8 level was reported to be associated with poor overall and disease-free survival in patients with hepatocellular carcinoma (HCC) after operation. We investigated the association of serum IL-8 levels and treatment response after transarterial chemoembolization (TACE) in patients with HCC.
Methods: A total of 119 patients with hepatitis B virus related HCC who underwent TACE were enrolled and followed up for TACE refractoriness and liver transplantation (LT)-free survival. Early TACE refractoriness was defined arbitrarily as TACE refractoriness within 12 months after the first TACE procedure. Pretreatment 11 serum cytokines levels (epidermal growth factor [EGF], fibroblast growth factor 2, granulocyte colony-stimulating factor [G-CSF], interferon-γ, IL-8, interleukin-12, interleukin-17A, interferon-γ-inducible protein-10, monocyte chemotactic protein-1, tumor necrosis factor-α and vascular endothelial growth factor) were analyzed using a Luminex 200 system (Millipore, Billerica, MA).
Results: During the mean follow-up period of 24.3 (1–79) months, 91 patients (76.5%) showed TACE refractoriness. Multivariate analyses showed that multiple tumor (Hazard ratio [HR], 2.37; 95% confidence interval [CI], 1.28–4.39; $P = 0.006$), large tumor size (HR, 2.36; 95% CI, 1.38–4.03, $P = 0.002$) and combination of AFP and IL-8 level (AFP >400 ng/ml or IL8 >32 pg/ml; HR, 1.72; 95% CI, 1.03–2.85, $P = 0.037$) are independent predictive factors for overall TACE refractoriness. Multiple tumor, large tumor size, high EGF level (>35 pg/ml; HR, 3.47; 95% CI, 1.01–11.96; $P = 0.049$), and low G-CSF level (<12.5 pg/ml; HR 6.25; 95% CI, 1.62–23.81; $P = 0.008$) were independent factors for prediction of early TACE refractoriness. High IL-8 levels were also associated with low probability of LT-free survival (HR, 1.68; 95% CI, 1.09–2.59, $P = 0.020$), together with multiple tumor number (HR, 4.43; 95% CI, 2.30–8.52, $P = 0.000$) and vascular invasion (HR, 2.24; 95% CI, 1.26–3.99, $P = 0.006$).
Conclusions: Pretreatment serum IL-8 levels are useful marker to predict overall TACE refractoriness and LT-free survival in patients with hepatitis B virus related HCC treated with TACE. Pretreatment serum EGF and G-CSF levels are also good markers for predict early TACE refractoriness in same patients.

P0257
LIPID PEROXIDES STIMULATE INTERLEUKIN-6 FORMATION FROM STELLATE CELLS BY AN OXIDATIVE STRESS-DEPENDENT MECHANISM

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Background and Aims: Lipid peroxides are intermediate oxidation products of unsaturated fatty acids formed under oxidative stress. Liver inflammation promotes oxidative stress which involves the tumorigenic factor interleukin-6 (IL-6). Perisinusoidal hepatic stellate cells play a key role in liver fibrosis with progression towards cirrhosis and hepatocellular carcinoma (HCC). We investigated the impact of lipid peroxides on IL-6 formation in stellate cells.
Methods: Human immortalized (LX-2) and primary human hepatic stellate cells were treated by various concentrations of native (LH) or peroxidized (LOOH) linoleic acid for different time periods with a maximal nontoxic effects at 20μM for 2 h. As a macrophage model, differentiated human monocytic THP-1 cells were used. Expression of IL-6 and C-reactive protein (CRP) was analysed by real-time RT-PCR and ELISA. Activation of IL-6 downstream targets Stat3 and p38 MAPK were investigated by western blotting.
Results: LOOH increased IL-6 expression and intracellular reactive oxygen species (ROS) in both immortalized and primary stellate cells. In particular, IL-6 increased from 2.07+0.66 pg/ml to 7.13+0.23 pg/ml in LX-2 cells ($p = 0.005$). In contrast, LOOH did not stimulate IL-6 in macrophages, whereas LPS was active in both cell types. Antioxidants N-acetylcystein and selenium as single agents or in combination inhibited the formation of ROS and LOOH-induced IL-6. PD169316, an inhibitor of p38 MAPK, prevented IL-6 upregulation by LOOH. Furthermore, when IL-6 containing supernatants from LOOH-treated LX-2 cells were applied to cultured human HCC-1.2 and HCC-3 cells, an IL-6 dependent phosphorylation of the transcription factor Stat3 as well as an increased expression of the downstream target CRP was observed. In particular, CRP mRNA and protein were increased in HCC-3 cells by 48+9%, $p = 0.0003$, and by 84+44%, $p = 0.013$, respectively.
Conclusions: These results suggest a novel mechanism of upregulation of pro-inflammatory IL-6 in stellate cells by lipid peroxides in a ROS-dependent way that can be inhibited by antioxidants.
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P0258
TAUROURSODEOXYCHOLIC ACID DAMPENS ONCOGENIC APOPTOSIS INDUCED BY ENDOPLASMIC RETICULUM STRESS DURING HEPATOCARCINOGEN EXPOSURE

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Background and Aims: Hepatocellular carcinoma (HCC) is characterized by accumulation of unfolded proteins in the endoplasmic reticulum (ER) which activates the unfolded protein response (UPR). However, the role of ER stress in tumour initiation

and progression remains a contentious topic. To determine the impact of ER stress, we applied tauroursodeoxycholic acid (TUDCA), a cytoprotective bile acid with chaperone properties reducing ER stress.
Methods: The effect of TUDCA on hepatic tumour burden, apoptosis, ER stress, autophagy, oxidative stress and inflammation was assessed in the diethylnitrosamine-induced orthotopic mouse model for HCC in a preventive and therapeutic setting. The effect on MTT metabolic activity and BrdU incorporation was investigated *in vitro* and the effect on tumour progression was assessed in a HepG2 xenograft model.
Results: Administration of TUDCA in a preventive setting reduced the carcinogen-induced elevation of aspartate and alanine aminotransferase levels and apoptosis of hepatocytes and, surprisingly, also the tumour burden. TUDCA markedly reduced the phosphorylation of eukaryotic initiation factor 2α, the expression of the pro-apoptotic C/EBP homologous protein (CHOP) and caspase-12 activation in the DEN-treated livers, suggesting that TUDCA suppressed terminal UPR signalling. Although no significant effects were observed on oxidative stress or autophagic flux, TUDCA alleviated hepatic inflammation associated with repeated carcinogen exposure. Furthermore, TUDCA dose dependently enhanced the cellular MTT metabolic activity, but not the cell proliferation rate *in vitro*. Importantly, administration of TUDCA in a therapeutic setting after tumour development did not affect the growth of orthotopic tumours or HepG2 xenografts.
Conclusions: These findings indicate that TUDCA attenuates hepatocarcinogenesis by suppression of carcinogen-induced ER stress-mediated cell death and inflammation without stimulation of tumour progression. Therefore, the chemical chaperone TUDCA could represent a novel chemopreventive agent for HCC.

P0259
TRANSENDOTHELIAL MIGRATION DEPENDS ON TRANSFORMING GROWTH FACTOR BETA IN HEPATOCELLULAR CARCINOMA PROGRESSION

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Background and Aims: Transendothelial migration of malignant hepatocytes into blood vessels represents a hallmark of hepatocellular carcinoma (HCC) progression. Overcoming of endothelial barrier is necessary for the entry into and exit from the circulation, termed intra- and extravasation. Yet, both mechanisms are poorly understood due to lack of appropriate study tools. We established a model of transendothelial migration by employing malignant hepatocytes, which have undergone epithelial to mesenchymal transition (EMT) dependent on transforming growth factor (TGF)-β and liver sinusoidal endothelial cells. The aim was to study the molecular mechanisms of transendothelial migration of EMT-transformed hepatocytes through hepatic sinusoidal blood endothelial barriers.
Methods: Murine liver sinusoidal endothelial cells (mLSECs) were isolated by liver perfusion from $p19^{ARF-/-}$ mice. Genomic stability of LSECs was examined by flow cytometry and their functional properties were assessed by tube formation and response to anti-angiogenic drugs. Integrity and polarity of endothelial cell layers were determined by transendothelial electrical resistance and confocal immunofluorescence analysis, respectively. Proteome profiling using stable isotope labeling with amino acids (SILAC) and mass spectrometry was performed to analyze real-time changes in protein expression during transendothelial migration.
Results: Lack of $p19^{ARF}$ allows immortalization of genetically stable LSECs expressing the endothelial markers VEGFR-2, VE-cadherin and CD146. LSECs show VEGF-induced tube formation and apoptosis after administration of the VEGFR inhibitors sorafenib or sunitinib. In addition, LSECs grow to compact monolayer with functional cell junctions including cell polarity. To study transendothelial migration, SILAC-labeled EMT-transformed hepatocytes were allowed to cross the layer of LSECs that were seeded on the bottom of collagen coated Transwell inserts. Expression of SILAC labeled proteins derived from either post-EMT hepatocytes and from SILAC-labeled LSECs was profiled in kinetics of endothelial transmigration in the presence and absence of TGF-β. Candidate regulatory proteins were further validated for their functional impact in endothelial transmigration.
Conclusions: We established a novel cellular model to accurately analyze the intravasation of EMT-transformed hepatocytes through the liver sinusoidal blood endothelium in a TGF-β dependent fashion.

P0260
LIVER KINASE B1 AS AN ONCOGENIC DRIVER IN LIVER CANCER

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Background and Aims: The liver kinase B1 (LKB1) is usually described as a tumor suppressor gene in a variety of tissues and is known to act as an upstream activator of AMP-activated protein kinase (AMPK), a central metabolic sensor. Surprisingly, recent evidence has underscored the role of LKB1 upregulation in advanced hepatocellular carcinoma as well as the therapeutic potential of LKB1 silencing in liver tumors. However, the controversial role of LKB1 in hepatocarcinogenesis initiation remains to be unequivocally identified.
Methods: Gene array, metabolomics profiling approaches were used on mice livers and hepatoma cells overexpressing LKB1. Western blot and qPCR analysis were also used to further investigate the effect of LKB1 overexpression.
Results: LKB1 overexpression accounts for deregulated cell proliferation and an overall malignancy-related phenotype with induction of carcinogenesis hallmarks. Under these conditions, LKB1 induces Ras guanyl-releasing protein 3 (RasGRP3) transcriptional upregulation, activating both the Ras/ERK and the mammalian target of Rapamycin (mTOR) pathways. LKB1-mediated Ras activation induces the elevation of Cyclin D1 levels, a master cell cycle regulator. Furthermore, induced mTOR activity promotes glycolysis and expression of pyruvate kinase muscle 2 expression in association with histone 3 phosphorylation and acetylation, which are essential for the induction of proliferation markers such as Cyclin D1. Interestingly, LKB1 overexpression is not associated with AMPK activation, suggesting a LKB1/AMPK uncoupling due to their cellular delocalization.
Conclusions: We provide strong evidence that LKB1 in the liver not only plays a role in liver cancer progression but is also an oncogenic driver and therefore a potential early diagnosis marker as well as a therapeutic target.

P0261

A GLOBAL RISK GENE SCORE PREDICTS EARLY AND LATE TUMOR RECURRENCE AFTER RESECTION OF HEPATOCELLULAR CARCINOMA

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Background and Aims: Patients undergoing treatment with curative intent for hepatocellular carcinoma (HCC) remain at high risk for tumor recurrence by the emergence of intrahepatic metastasis ("early recurrence") or the formation of new lesions ("late recurrence"). We aimed to develop and validate a gene expression signature that could predict this risk.
Methods: HepG2 liver cancer cells made resistant to sorafenib during several months were used as a model for hepatocyte dedifferentiation and aggressive tumor biology. The differential expressed genes between this cell line and its non-resistant parental lineage were determined using an Affymetrix microarray platform. To form a score, the number of genes was subsequently downsized by assessing the performance of each one in published microarray data sets of HCC samples (GSE25097, GSE9843) and samples of non-tumoral surrounding liver (GSE40873). This resulted in a 7 gene score (Global Risk Score, GRS) which was validated in two independent data sets (LEC cohort, GSE1898/GSE4024, n = 67 and NCI cohort, GSE14520, n = 247) using a tumor- and liver-specific cut-off for high and low GRS.

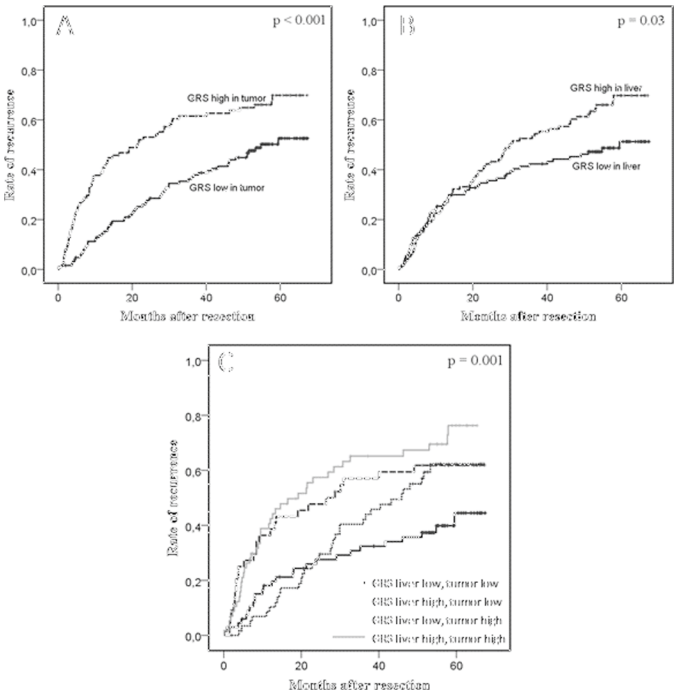

Figure 1. Performance of the Global Risk Score in the NCI cohort (n = 247).

Results: The GRS, when assessed in tumor tissue of patients treated with resection, identified patients at low and high recurrence risk at 3 years in the LEC (68±10% vs 35±7%, p = 0.03) and NCI (62±5% vs 37±4%, p < 0.001, Figure 1A) cohort. Moreover, assessment of the GRS in non-cancerous surrounding liver tissue of the NCI patients revealed that, starting at about 19 months after resection, the score was correlated with the formation of new lesions in the cirrhotic liver (Figure 1B). Taken together, based on the tumor and liver score value, the GRS identified 4 patient groups with differences in recurrence rates and time patterns of recurrence (Figure 1C). Multivariable analysis by Cox regression showed that GRS group was a significant predictor of recurrence, independent from BCLC stage (p = 0.007).
Conclusions: We present the first gene score validated in both HCC tissue and surrounding liver based on an in vitro model. By identifying aggressive tumor biology as well as tumorigenic potential of the underlying liver disease, the Global Risk Score allows calculating a prognostic estimate for an individual patient facilitating therapeutic decisions.

P0262

INHIBITION OF REGULATORY T CELLS USING THE FOXP3-INHIBITORY PEPTIDE P60 IMPROVES ANTITUMOURAL EFFECT OF A VACCINATION WITH mAFP-EXPRESSING DC IN SUBCUTANEOUS AND ORTHOTOPIC MURINE HCC MODEL

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Background and Aims: Dendritic cells (DC) are professional antigen-presenting cells able to prime T-cell responses against tumour-associated antigens such as alpha-fetoprotein (AFP). However, the antitumoural effect of DC vaccination in therapy of hepatocellular carcinoma (HCC) is limited by the immunosuppressive tumour environment. Immunosuppressive regulatory T-cells (Treg) are increased in tumour milieu and peripheral blood of patients with HCC. The aim of this study was to analyse whether a functional inhibition of Treg improves the antitumoural effect of a vaccination with murine (m) AFP-expressing DC in a subcutaneaous (s.c.) and an orthotopic murine HCC model.
Methods: DC were gained from bone marrow of C3H mice and transduced on day 6 with a mAFP-encoding adenovirus (Ad-mAFP). For tumour induction, 10^6 or 10^5 mAFP positive Hepa129 cells were injected s.c. into the right flank or into the left liver lobe of C3H mice respectively. On days 9 and 15 after s.c. or on day 3 and 6 after orthotopic tumour induction 10^6 Ad-mAFP-transduced or Ad-Mock-transduced DC were s.c. inoculated. In addition, mice were treated with a daily intraperitoneal (i.p.) injection of 100 μg of the FOXP3-inhibitory peptide P60 or the control peptide P301.
Results: A monotherapy with the FOXP3-inhibitory peptide P60 or with a vaccination using Ad-mAFP-transduced DC had no positive effect on the outcome of tumour-bearing mice (p > 0.05). In contrast, the combination of P60 and vaccination with mAFP-expressing DC lead to a significant tumour inhibition and prolonged survival in s.c. and orthotopic AFP-positive HCC model. Interestingly, the synergistic antitumoural effect of the combined therapy was stronger in the orthotopic HCC model (p = 0.0056). On day 21, 90% of the tumour bearing mice treated with P60 and mAFP-expressing DC and only 14% of the untreated mice with orthotopic HCC had survived. Even one week later, 40% of the therapy group still lived while all of the untreated mice had died.
Conclusions: We showed that the functional inhibition of Treg using the FOXP3-inhibitory peptide P60 or a vaccination with mAFP-expressing DC alone has no antitumoural effect towards HCC *in vivo*. However, P60 in combination with Ad-mAFP-transduced DC synergistically decelerates tumour growth improving the outcome of HCC-bearing mice. Thus, these results emphasize the potential

of the specific inhibition of Treg using P60 to enhance the antitumoural effect of DC vaccination in the therapy of HCC.

P0263
CONSEQUENCES OF β-CATENIN PATHWAY ACTIVATION ON MOUSE HEPATIC METABOLISM

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Background and Aims: Hepatocellular carcinoma (HCC) are the 3rd cause of cancer-related death in the world. 20 to 40% of HCC are caused by activating mutations in the CTNNB1 gene coding for β-catenin. They are poorly "proliferative", well differentiated and display a specific metabolic phenotype: indeed these tumors are cholestatic and never steatosic. Currently, tumor metabolism is a potential target in the treatment of cancer. The aim of this study was to determine the metabolic alterations leading to this distinctive metabolic phenotype in hepatic tumors where β-catenin is over-activated
Methods: We use the inducible liver-specific APC KO mouse models TTR-Cre-Apclox/lox. We either activate constitutively β-catenin in the whole hepatic lobule to mimic a "preneoplasic" situation or we abnormally activate β-catenin in few hepatocytes that will then generate tumors after 6 months. Using these two approaches we analyze metabolic fluxes to determine the carbon fate from different potential substrate in order to identify the source of energy and cellular components of β-catenin activated hepatocytes or tumors.
Results: Our results reveal that an abnormal activation of β-catenin in hepatocytes leads to an increase in fatty acids β-oxidation with a decrease in triglycerides synthesis which may explain the lack of steatosis. This has to be seen in connection with the loss of fat mass in our "preneoplasic" model that indicates an increase in lipid reserve utilization. Regarding tumor metabolism, Warburg effect, an aerobic glycolysis, is often described as many tumors use glucose as a preferential substrate. Here we show that there is no increase in the utilization of glucose in β-catenin activated hepatocytes but a rerouting of its carbons towards ribose synthesis instead of glycolysis or mitochondrial oxidation. Moreover the mouse β-catenin tumors are negative in PET-scan FDG and metabolic flux analyses revealed that they oxidize fatty acids much more efficiently than the adjacent normal tissue.
Conclusions: Our results show that an abnormal activation of the β-catenin pathway in hepatocytes leads to a reorientation of liver metabolism. Hepatocytes and tumors then use fatty acids as the main energy source and glucose is rerouted into the pentose phosphate pathway.

P0264
REDIRECTING ADENOVIRUS-SPECIFIC T CELLS BY A TUMOR-SPECIFIC T CELL RECEPTOR FOR THERAPY OF HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma is highly resistant to conventional therapies and need alternative treatments. Oncolytic viruses are promising tools for the treatment for solid tumors but unfortunately trigger strong T cell immune responses against the applied virus.
Here, we wanted to investigate whether antiviral cytotoxic T lymphocytes triggered by oncolytic virotherapy can be redirected with a tumor specific TCR for therapy of HCC in an immunocompetent C57BL/6 mouse model.
Methods: First, virus-specific T cells were induced by intraperitoneal challenge with oncolytic Adenovirus in Thy1.1 mice and splenocytes were used to generate bispecific T cells by lentiviral transfer of the OT-1 receptor in adenovirus-specific T cells. Resulting bispecific T cells were adoptively transferred into Thy1.2 recipient mice bearing HCC tumors expressing OVA minimum epitopes. Prior to adoptive transfer, mice were preconditioned with Cyclophosphamid and Treosulfan to achieve lymphoreduction.
Results: To obtain a suitable HCC cell line for a syngeneic tumor model we used the transposon system. We hydrodynamically injected an oncogenic transposon coding for KRasG12V/myrAkt1 and a transposon for MHC class I and II minimum epitopes of OVA, SB13, and Cre recombinase in p53fl/fl mice. FACS analyses on isolated tumor clones showed that the cell line LV13531 constitutively expressed MHC class I which can be further triggered by IFN-gamma. The cell line was used for inoculation of subcutaneous tumors. After adoptive transfer of bispecific T cells, tracking lentiviral encoded GFP and CD8 by FACS analysis, proved successful establishment of transduced CD8 T cells in blood, spleen and tumor tissue. Finally we demonstrated that, compared with naïve and immunized mock-transduced cohorts, the group which received Ad/OT-1 specific T cells showed significantly improved regression and long-term survival.
Conclusions: Our findings suggest that virus-specific T cells induced by oncolytic virotherapy can be successfully redirected with a tumor specific TCR to address therapy-resistant HCC.

P0265
IGF2 IS AN ONCOGENIC DRIVER IN HCC AND EMERGES AS A POTENTIAL TARGET FOR THERAPIES

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Background and Aims: Hepatocellular carcinoma (HCC) is the 16th absolute cause of death world-wide and accounts for 90% of all liver cancers. IGF-Insulin signaling has a relevant role in HCC pathogenesis and elucidation of its key molecular drivers is important to overcome the poor therapeutic results obtained so far by inhibiting this pathway. Since IGF2 ligand have been shown to be overexpressed in HCC, we aimed (1) to explore the oncogenic potential of IGF2 in genetically modified animal models (GEMM), (2) to elucidate the oncogenic mechanism of IGF2 through the receptors IGF1R and IR, and (3) to determine the antitumoral efficacy of molecular therapies against this target.
Methods: A GEMM overexpressing IGF2 in the liver was generated using hydrodynamic tail vein injection. A cohort of 228 human HCCs was characterized analyzing gene expression, exomic mutations, DNA copy number and methylation status with a focus on the IGF-Insulin pathway. Activation of IGF2 promoters and expression of isoforms A and B from IR were additionally assessed by qRT-PCR. Therapeutic potential of the monoclonal antibody BI836845 (Boehringer Ingelheim), selective for IGF ligands, was studied in

HCC cell lines by analyzing cell viability, proliferation and pathway activation.
Results: Only mice overexpressing IGF2 showed increased number/size of tumors and significantly reduced survival ($p = 0.02$) when compared to control mice. In humans, 15% (31/207) of patients in our cohort showed IGF2 overexpression (FC >2), being hypomethylation of fetal promoters the main cause in 87% of cases (21/54 vs 7/171, $p < 0.0001$). Patients with high levels of IGF2 showed significant association with stem-cell signatures. In addition, 40% of the HCCs overexpressed the IR-A (which has a pro-proliferative input, in contrast with the pro-metabolic input caused by IR-B). In cell lines with increased levels of IGF2 and IR-A, selective IGF-ligands blockade with BI836845 significantly reduced cell viability and proliferation (30%, $p = 0.002$), inhibiting IGF-Insulin pathway activation, and without interfering with insulin metabolic effect.
Conclusions: Overexpression of IGF2 has a relevant role in the process of hepatocarcinogenesis through activation of IGF1R and IR-A in a subgroup of HCC patients. These results provide the rationale that favors therapeutic strategies based on monoclonal antibodies against IGF ligands, in front of tyrosine kinase inhibitors, to selectively block the pathway.

P0266
ADENO-ASSOCIATED VIRUS 2 (AAV2) INDUCES RECURRENT INSERTIONAL MUTAGENESIS IN HUMAN HEPATOCELLULAR CARCINOMAS

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Background and Aims: Hepatocellular carcinomas (HCC) are related to various etiologies including hepatitis B and hepatitis C virus infection, alcohol intake, obesity, hemochromatosis. Additional risk factors remain to be identified particularly in HCC developed on non-fibrotic liver. We identified a fragment of Adeno-Associated Virus type 2 (AAV2, a defective DNA virus considered as non-pathogenic) inserted in an HCC genome and this result prompted us to screen a large series of HCC samples.
Methods: We screened 150 HCC and matched non-tumor liver samples for AAV2 sequences using PCR and next generation sequencing. Expression of the genes targeted by viral integration was quantified and the functional consequences of AAV2 integration within TERT promoter was analyzed in 3 HCC cell lines using luciferase assay.
Results: Among the 150 tumors, we identified 7 cases (5%) with a clonal AAV2 insertion whereas no clonal insertion was found in the corresponding non-tumor liver tissues. In the first HCC tumor, we identified a 208 base pairs insertion of an AAV2 fragment within the TERT (coding for the telomerase reverse transcriptase) promoter. In HCC cell lines, we demonstrated that this AAV2 insertion resulted in an increased TERT promoter activity. We next identified 6 additional clonal somatic integrations of AAV2, all occurred within known cancer driver genes, CCNA2 (4 cases), CCNE1 or TNFSF10 leading to over-expression of the targeted gene without generating chimeric viral-endogeneous functional fusion transcripts. In CCNA2 (coding for cyclin A2), the four integration sites were identified within intron 2 and AAV2 insertion ranged from 219 to 1,975 bp occurring in both orientations. In CCNE1 (coding for cyclin E1), we identified a 368 bp AAV2 integration within intron 4. In the remaining case, AAV2 integration occurred in the 3′ UTR of TNSFS10 (coding for the receptor TRAIL), 244 bp after the stop codon. In 6 out of the 7 tumors, the inserted AAV2 included the 3′ inverse tandem region. Strikingly, almost all the tumors were developed on non-fibrotic liver (6 out of 7 cases, $p < 0.05$) without known risk factor (5 out of 7, $p < 0.05$), thus suggesting a particular pathogenic role of AAV2 in this subset of patients.
Conclusions: Infection by AAV2, that is very frequent in the general population (around 30–60% of individuals), is involved in the pathogenesis of rare human HCC by recurrent somatic integration in cancer driver genes challenging its safety as a vector for gene therapy.

P0267
RAS AND P-ERK IN METABOLIC SYNDROME ASSOCIATED HEPATOCELLULAR CARCINOMA (HCC)

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Background and Aims: HCC mortality associated with the metabolic syndrome (MetS) is rising, often in the absence of liver cirrhosis. We reported an animal model in which C3H/He mice with impaired glucose tolerance, fed the American lifestyle (ALIOS) diet, develop NASH, fibrosis and HCC at 1 year. While activated Ras/MAPK signalling is often key in murine carcinogenesis, increased p-ERK is reported in just 2.5–10% of HBV/HCV associated human HCC. We have assessed this pathway in our animal model and in MetS HCC.
Methods: H&E sections, DNA, RNA and protein from paired murine tumour and non-tumour (NT) livers were compared. p-ERK was with western blot in mice and immunohistochemistry in paraffin embedded needle biopsies from 20 MetS HCC patients without any other contributing liver disease. *KRAS* mutations in plasma DNA were explored using a MALDI-TOF platform.
Results: Murine development of large HCC (6–15 mm, with nuclear atypia and loss of reticulin) was associated with histologically confirmed NASH in ALIOS fed mice. Marked microvesicular steatosis, ballooning and inflammation were common in the tumours, which were classed as steatohepatitic HCC (SH-HCC). AFP and glypican-3 were up-regulated over 100 fold, with iNOS (1.22 ± 0.31 vs. 14.25 ± 5.23; $p = 0.001$) and TNFα (0.63 ± 0.08 vs 1.66 ± 0.54, $p = 0.007$) also elevated in tumours. *H-ras* mutation at codon 61 was present in 5/20 (25%) of HCC, while p-ERK was up-regulated relative to paired NT in all cases (5.5 ± 0.6 vs 99.8 ± 26.0, $p = 0.004$). In 20 biopsied MetS HCC patients, 35% had cirrhosis and 80% had NAFLD. Three (15%) HCC had minimal (<5%) p-ERK positive nuclei assessed by immunohistochemistry and digital quantification (Aperio Imagescope). Of 17 HCC with >10% nuclei positive for p-ERK, in 6 (30%) this was in excess of 50% nuclei (none cirrhotic, all diabetic, BMI median 24.8). In 11 (55%) with SH-HCC, p-ERK was classed as moderate (10–35% nuclei; 8 cirrhotic; 9 diabetic; BMI median 32.0). Plasma DNA *KRAS* mutations in codon 12 were detected in 2 patients.
Conclusions: Alteration of the Ras/MAPK pathway was evident in 85% of metS associated Human HCC, including marked p-ERK elevation in 30% (actually 50% of non-cirrhotic cases). A further 55% of HCC had moderately elevated p-ERK, in association with SH-HCC features. *KRAS* mutations in circulating plasma DNA were detected in 10%. The C3H/He ALIOS model will be useful for studying Ras/MAPK in the pathogenesis of these tumours. Treatments targeting this pathway may be particularly relevant for patients with MetS associated HCC.

P0268

ACCUMULATION OF DELETERIOUS PASSENGER MUTATIONS DURING LIVER INJURY PROGRESSION TO HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma (HCC) is associated with genetic alterations in several driver genes. However, no single driver mutation is necessary or sufficient for carcinogenesis. The vast majority of mutations unique to the patients, known as passenger mutations, have been so far ignored. Some passenger mutations are deleterious and could act as markers for disease progression. A previous study has shown that deleterious passenger mutations (DPMs) occur more frequently in tumour tissue compared to surrounding non-tumour tissue. The accumulation of DPMs was hypothesised to be due to: (1) that DPMs occur more frequently than driver mutations; and (2) the accumulated DPMs eventually offset the survival advantage conferred by driver mutations. Therefore, we aim to detect DPMs in liver disease progression and hypothesise that DPMs accumulate in hepatocytes in precancerous liver disease leading up to HCC.
Methods: We have performed whole exome sequencing of pre-cancerous liver tissues: 12 patients with limited level of liver injury and 6 HCV-positive patients with liver cirrhosis. We have also analysed publically available paired HCC and surrounding non-tumour datasets. To compare different data we only analysed mutations that occurred in protein-coding genes, filtered out probable germline mutations and normalised the analysis to only exonic variants detected. Poly-Phen2 was employed to predict impact of variants on protein function.
Results: We have found that HCC genomes contain significantly more DPMs compared to paired non-tumour tissue (33.1 vs 30.3% total exonic variants respectively, $p < 0.001$ by paired two-tailed t-test). Comparison with non-cirrhotic and cirrhotic tissues showed significantly fewer DPMs (19.9 and 21.7% respectively) compared to tumour and non-tumour tissues. Moreover, we observed a significantly more DPMs in non-tumour tissue as the number of putative driver genes with damaging mutations increases (24.1, 31.7, 34.8% for 0, 1, ≥2 drivers respectively). DPMs were evenly distributed across the genome and the majority were unique for each patient, suggesting they are truly passenger mutations.
Conclusions: We showed that DPMs accumulate in precancerous tissues leading up to HCC. Moreover, precancerous alterations were found in non-tumour tissue, despite being used in prior studies as normal paired controls. We are currently sequencing larger patient cohorts with precancerous states to develop marker regions that predict HCC risk.

P0269

THE INSULINE-LIKE GROWTH FACTOR 2 (IGF2) MRNA BINDING PROTEIN (IMP) P62 PROMOTES CIRRHOSIS-LINKED HEPATOCARCINOGENESIS

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Background and Aims: The insulin-like growth factor 2 (*IGF2*) mRNA binding protein (IMP2) p62 was shown to accelerate progression of NASH to a disease stage with fibrosis (Simon et al. *Gut*, 2014). Aim of our study was to test the hypothesis that p62 promotes fibrosis progression towards malignancy.
Methods: NASH was induced by feeding either a methionine-choline deficient (MCD) or a control diet in wild-type or *p62* transgenic mice for 2 and 4 weeks. Carcinogenesis was induced by diethylnitrosamine (DEN, 5 mg/BW *i.p.*) given either after or prior to the onset of MCD feeding (up to 21 weeks). Tissues were histologically analyzed by HE and Sirius red staining. mRNA and protein levels were investigated by real-time RT-PCR and immunohistochemistry, respectively.
Results: On normal chow *p62* transgenic animals showed elevated expression of *E-cadherin*, an epithelial marker overexpressed in liver progenitor cells (LPC). In MCD-induced NASH a strong ductular reaction and LPC proliferation was observed in *p62* transgenic mice. Gene expression of laminin, an extracellular matrix (ECM) component supporting LPC proliferation, increased in MCD-fed *p62* transgenic animals. Expression of hyaluronic acid receptor was not altered. Expression analysis revealed that *p62* transgenic mice have specifically increased *Krt19* expression. *Sox9*, another biliary marker of the ductal plate, was induced in both genotypes upon MCD feeding. Immunohistochemical characterization rvealed small nests of LPC as well as bile duct-like formations. LPC proliferation was surrounded by an ECM layer and directed into the lobule by ECM deposition ahead of the LPC.
When carcinogenesis was induced by injection of DEN after the onset of MCD feeding, all animals died within 10 weeks or had to be sacrificed; survival was significantly reduced in *p62* transgenic animals ($p = 0.0056$). Administration of DEN before the onset of MCD feeding (21 weeks) induced macroscopically visible hyperplastic nodules preferentially in *p62* transgenic mice ($p = 0.0019$). Sirius red staining confirmed cirrhotic changes solely in the livers of *p62* transgenic animals. Interestingly, *p62* transgenic mice overexpressed the hepatocellular carcinoma (HCC) marker alpha-fetoprotein (AFP) mostly in hepatocytes and rarely in LPC suggesting carcinogenic changes in the cirrhotic tissue.
Conclusions: p62 promotes a progression of fibrosis towards cirrhosis and thus promotes hepatocarcinogenesis.

P0270

SERPINB3 AND YAP INTERPLAY INCREASES MYC ONCOGENIC ACTIVITY

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Background and Aims: SerpinB3 has been recently described as an early marker of liver carcinogenesis, but the potential mechanistic role of this serpin in tumour development is still poorly understood. Myc is one of the most important oncogenes in human cancer. Somatic amplification and overexpression of Myc often correlate with more advanced and aggressive tumour forms, supporting its involvement in carcinogenesis. Yes-associated protein (Yap), the main effector of the Hyppo pathway, is a central regulator of proliferation and self-renewal of normal and cancer stem cells. This molecule, found up-regulated in hepatocellular carcinoma (HCC), has been described also to increase Myc expression.
The present study has been designed in order to investigate whether SerpinB3 may functionally modulate Myc in different experimental models and in human HCC specimens.
Methods: Expression of Myc, Yap and its target genes was evaluated in relation to SerpinB3 expression in HCC specimens from 67

patients obtained at the time of surgical resection, in C57BL/6 mice transgenic for human SerpinB3. In addition, mRNA quantification and Western Blot analysis was performed in HepG2 and Huh7 cells stably transfected with plasmid vectors carrying the SerpinB3 gene. Moreover the inhibitory effect of SerpinB3 on calpain was assessed by means of the calpain activity assay.

Results: A positive correlation between Myc and SerpinB3 expression was observed at transcription and protein level in HCC specimens, where Myc oncogene was found predominantly in the nucleus of cancer cells overexpressing SerpinB3. Myc expression was significantly and mechanistically up-regulated by SerpinB3 through calpain and Hyppo-dependent molecular mechanisms. Recombinant SerpinB3 protein was indeed capable to inhibit the activity of calpain in vitro, likely reducing its ability to cleave Myc in its non-oncogenic Myc-nick cytoplasmic form. Furthermore, SerpinB3 indirectly increased the transcription of Myc through the induction of Yap pathway, as documented by a remarkable Yap nuclear translocation and up-regulation of Yap target genes in transgenic mice and in hepatoma cells overexpressing human SerpinB3.

Conclusions: Data from the present study provide evidence that SerpinB3 can improve the production of Myc oncogene through direct and indirect mechanisms that include the inhibition of generation of its cytoplasmic form and the activation of Yap pathway.

P0271

EFFECTS OF FXR AGONIST PX20606 ON TUMOUR FORMATION IN ANIMAL MODELS OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is an increasingly common cause of cirrhosis and hepatocellular carcinoma (HCC). FXR receptor agonists have shown promise, reducing steatosis, inflammation and fibrosis. We explored the effects of the FXR receptor agonist PX20606 on tumour formation in two murine models of NAFLD.

Methods: C57BL/6 mice fed high-fat diet (HFD) plus 1% cholesterol received 0, 1, 3 or 10 mg/kg PX20606 in food for 12–18 weeks (n = 17, 7, 8, 17 respectively). In a longer term study, C3H/He mice (Harlan), which develop obesity, fatty liver and HCC with age, were fed American lifestyle (ALIOS, n = 24) or control diet (n = 23) from 8 until 48 weeks of age. Parallel groups received PX20606 1 mg/kg (n = 24) or 5 mg/kg (n = 16) for the final 24 weeks.

Results: In C57BL/6 mice at 12–16 weeks, plasma cholesterol and liver triglycerides were suppressed by PX20606, with no significant changes in ALT. Surface liver tumours (2–3 mm) were evident after 18 weeks of PX20606 treatment in 9%, with no significant difference between groups (Table 1A). In C3H/He mice at 48 weeks, PX20606 lowered the total cholesterol/HDL and TG levels (Table 1). While all C3H/He mice developed NAFLD with age, ALIOS diet markedly exacerbated this, with increased steatosis, ballooning, lobular inflammation, fibrosis and with a greater number and size of HCC (Table 1B). PX20606 modestly reduced the histological severity of NAFLD in ALIOS fed animals, but had no impact on tumour number or size. Unexpectedly, on the control diet, the severity of hepatitis, as well as tumour number and size increased stepwise with PX20606 dose (Table 1B). In these animals, tumour development was strongly associated with liver weight (Spearman 0.425, p = 0.005) and hepatocellular ballooning (Spearman 0.491, p = 0.001).

Table 1A. C57BL/6 mice (12–16 weeks) fed HFD with or without FXR agonist PX20606

PX20606	0 mg	1 mg	3 mg	10 mg	p (10 mg to 0 mg)
Number of mice	17	7	8	17	
HCC mean	0.23±0.11	0.14±0.14	0	0.18±0.10	0.531
Liver (g)	3.30±0.93	2.71±1.04	3.08±0.64	2.90±0.60	0.118
ALT	90.41±43.31	65.00±32.98	59.12±31.62	81.88±56.67	0.625
Total CH	277.71±58.22	129.00±45.64	138.13±43.03	81.76±29.33	0.000
Liver TG (μg/mg)	123.01±14.58	101.05±2.36	108.18±4.21	43.80±13.72	0.000

Conclusions: Treatment with FXR agonist PX20606 strongly affected lipids in C57BL/6 and modestly affected lipids and NASH in C3H/He, but did not reduce HCC risk in either animal model of NAFLD. In 18 weeks C57BL6 HFD mice or 48 weeks C3H/He ALIOS mice, there were no significant differences in HCC incidence associated with PX20606 treatment. However, in 48 weeks control diet C3H/He mice with background mild NAFLD, HCC risk increased stepwise with PX20606 dose, associated with increased liver weight and hepatocellular ballooning. These data may not be an FXR-agonist class effect and could reflect a rodent or strain-specific mechanism. However, studies assessing similar agents as long-term treatments in humans should consider HCC risk.

Table 1B (abstract P0271). C3H/He mice (48 weeks) fed control or ALIOS diet, with or without FXR agonist PX20606

	Control diet				ALIOS diet				p 6 groups (KW)
PX20606	0 mg	1 mg	5 mg	p	0 mg	1 mg	5 mg	p	
Number	23	12	8	(KW)	24	12	8	(KW)	
Total/HDL	1.59±0.06	1.62±0.08	1.33±0.01	0.002	1.66±0.08	1.45±0.05	1.29±0.02	0.002	0
TG	1.43±0.13	1.32±0.11	1.11±0.17	0.409	1.39±0.19	1.06±0.17	0.78±0.10	0.068	0.041
Liver (mg)	1.85±0.06	2.32±0.11	2.81±0.12	0	2.84±0.19	3.47±0.15	3.30±0.14	0.001	0
Steatosis grade	1.27±0.13	2.00±0.30	2.00±0.38	0.072	2.53±0.12	2.17±0.11	2.29±0.18	0.121	0
Lobular Inflam	0.59±0.16	0.92±0.19	1.0±0.00	0.121	1.40±0.14	1.0±0.17	1.00±0.21	0.17	0.014
Ballooning	0.91±0.13	1.25±0.27	1.63±0.26	0.049	1.58±0.14	1.58±0.15	1.14±0.14	0.117	0.01
NAS score	2.73±0.30	4.08±0.42	4.63±0.56	0.006	5.53±0.31	4.75±0.22	4.42±0.48	0.069	0
ALP	51.5±2.5	60.6±3.43	101.5±4.48	0	69.7±4.46	93.4±4.86	111.8±6.10	0	0
Albumin	27.18±0.59	28.74±0.65	28.72±0.84	0.099	29.43±0.74	31.65±1.26	32.00±0.94	0.081	0.001
Bilirubin	4.24±0.26	3.44±0.35	3.69±0.62	0.092	4.92±0.92	3.71±0.30	3.05±0.47	0.12	0.099
ALT	37.9±11.01	48.16±6.57	163.25±60.4	0.003	49.09±8.39	51.27±3.77	79.66±11.77	0.003	0
HCC									
mean	0.30±0.10	2.17±1.21	2.50±1.16	0.028	2.04±0.48	1.33±0.31	2.62±0.73	0.248	0
size	0.96±0.43	1.77±1.18	4.57±1.38	0.042	7.65±1.63	7.63±2.01	6.31±2.16	0.967	0

P0272

LONG-TERM INHIBITION OF EGFR IN HUMAN CHOLANGIOCARCINOMA CELLS LEADS TO THE INDUCTION OF AN EPITHELIAL TO MESENCHYMAL TRANSITION PROGRAM AND ACTIVATION OF INSULIN/INSULIN GROWTH FACTOR 1 RECEPTORS

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Background and Aims: Cholangiocarcinoma (CCA) is a liver cancer with poor prognosis due to local invasion and metastasis. Epidermal growth factor receptor (EGFR) is overexpressed in CCA tumors and plays a major role in CCA progression. Thus, EGFR has been envisaged as molecular target for CCA therapy. However, clinical trials using anti-EGFR such as the small-molecule tyrosine kinase inhibitor (TKI) erlotinib did not provide a therapeutic benefit in patients with CCA, suggesting resistance to anti-EGFR therapies. This study was designed to unravel the underlying cellular and molecular mechanisms involved in acquired resistance to anti-EGFR in CCA.
Methods: Cell pools resistant to erlotinib were obtained by treating four human CCA cell lines (HuCCT1, SK-ChA-1, EGI-I and Mz-ChA-1) with increasing concentrations of erlotinib (0–20 μM) for a long period of time (6–9 months). Cell viability was determined by MTT assay. Epithelial to mesenchymal transition (EMT) was investigated by examining the expression of epithelial and mesenchymal markers by RT-qPCR, western blot and immunofluorescence. Signaling pathways were analyzed by phosphoprotein arrays, immunoprecipitation and western blot.
Results: The four erlotinib-treated CCA cell lines showed reduced sensitivity to TKI toxicity compared to their parental counterparts. Long-term treatment with erlotinib induced CCA cells scattering, which was accompanied by a disruption of the adherens junctions as attested by the internalization and/or decreased expression of E-cadherin, as well as dephosphorylation and nuclear translocation of β-catenin. In pools with acquired resistance to erlotinib, the expression of EMT-transcription factors (SNAIL, SLUG and ZEB-1) and mesenchymal markers (*i.e.* N-cadherin and α-SMA) was induced. Additionally, the expression of cancer stem cell (CSC) markers (*i.e.* CD117, CD133 and ABCG2) was also up-regulated in erlotinib-resistant cells. In these cells, all members belonging to the ERBB family (ERBB1/EGFR, ERBB2, ERBB3 and ERBB4) were inhibited. In contrast, insulin/insulin growth factor 1 receptors (IR/IGF1R) were activated and IGF2, a ligand for IR/IGF1R, was overexpressed in resistant cells compared to untreated cells.
Conclusions: The establishment of an EMT program with the acquisition of CSC phenotype, and the activation of the IGF signaling axis could contribute to the resistance of CCA cells to erlotinib.

P0273

ISCHEMIC PRECONDITIONING OF INJURED STEATOTIC LIVERS REDUCES HEPATOCELLULAR CARCINOMA RECURRENCE

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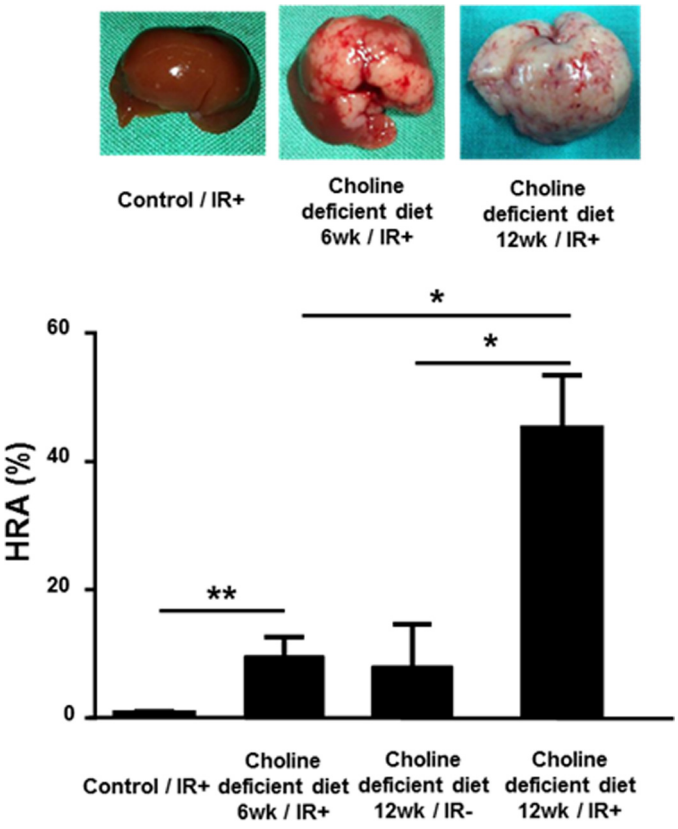
Background and Aims: Although livers with parenchymal abnormalities poorly tolerate ischemic damage, there is limited data as to whether the susceptibility of steatotic livers to ischemia-reperfusion (IR) injury also impacts on cancer recurrence.
Methods: Wild type C57BL/6 mice were fed with a choline-deficient (CD) diet for 6 and 12 week, or standard chow. Hepatic IR injury and ischemic preconditioning were achieved by clamping the liver blood inflow. Hepa 1–6 hepatocellular carcinoma (HCC) cells were inoculated through the spleen. After three weeks, tumor burden, serum alpha fetoprotein and cancer cell aggressiveness were compared among groups.
Results: Hepatocellular damage and inflammatory genes (*Il6, Tnf-α, Hif-1α, E-selectin*) expression were significantly exacerbated after IR injury in severely steatotic mice. Compared to control livers or those with minimal steatosis, livers exposed to prolonged CD diet developed larger tumor nodules, and exhibited higher serum AFP levels. Non-ischemic lobes of steatosis/IR+ mice were not protected from IR-mediated accelerated tumor overgrowth. This remote effect was linked to a promotion of the aggressiveness of HCC cells exposed *in vitro* to the serum of steatosis/IR+ mice. Importantly, the tumor burden of livers undergoing ischemic preconditioning before IR was reduced to the level of non-ischemic steatotic controls.
Conclusions: Steatotic livers poorly tolerate IR injury, contributing to more severe HCC recurrence patterns in mice with increasing degree of fatty liver infiltration. IR mitigation by performing ischemic preconditioning results in reduced tumor load and serum AFP. In addition to *in situ* effects, the IR-related susceptibility of steatotic livers impacts on remote cancer cell aggressiveness.



P0274

LIVER TUMOR INCIDENCE IS DEPENDENT ON SEX AND TELOMERASE STATUS IN TRP53 KNOCKOUT MICE

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Background and Aims: Key features of human liver cancer are telomere shortening, dysregulation of the p53-signaling pathway and chromosomal instability. Defects in the p53-signaling pathway

are frequently associated with increase in stem cell features, mixed differentiation and poor prognosis. In this study we analyze the cooperation between telomere dysfunction and p53 loss in a humanized mouse model.
Methods: We crossed telomerase knockout mice (Terc$^{-/-}$) with a conditional Trp53 mouse and a liver-specific AlfpCRE mouse (CRE expression under the albumin-alphafetoprotein-enhancer-promoter) to induce a liver-specific deletion of the Trp53 gene during embryogenesis. We crossed Terc$^{-/-}$ mice up to the third generation (G3 Terc$^{-/-}$) for the generation of short telomeres. At this step we intercrossed G3 Terc$^{-/-}$ with Terc$^{+/-}$ mice. Through this mating strategy we produced Trp53 knockout mice lacking telomerase (AlfpCre, Trp53Δ/Δ, iG4 Terc$^{-/-}$) and mice with reactivated telomerase (AlfpCre, Trp53Δ/Δ, iF1 Terc$^{+/-}$). We compared these mice with Trp53 knockout mice with long telomeres and active telomerase (AlfpCre, Trp53Δ/Δ, Terc$^{+/+}$).
Results: Mice of all three cohorts developed liver cancer with an incidence of up to 80% showing a mixed tumor differentiation of hepatocellular carcinoma and cholangiocellular carcinoma (HCC-CC). Tumor formation frequently occurs in male mice. The tumor incidence was slightly increased in male mice with short telomeres and lack of telomerase. Liver tumor formation in female mice was a rare event at the age of 12–14 month but increases in females with short telomeres and telomerase reactivation (AlfpCre, Trp53Δ/Δ, iF1 Terc$^{+/-}$: 6 out of 11; AlfpCre, Trp53Δ/Δ, iG4 Terc$^{-/-}$: 2 out of 18; AlfpCre, Trp53Δ/Δ, Terc$^{+/+}$: 2 out of 12).
Conclusions: Overall this study shows that Trp53 deletion frequently results in liver tumor formation in male mice, whereas incidence of tumor formation in female mice depends on telomere length and telomerase status. The arising liver tumors show an increase in stem cell features.

P0275
PDGFRA-MEDIATED LAMININ B1 DEPOSITION INDUCES INVASION IN HUMAN HEPATOCELLULAR CARCINOMA
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Background and Aims: Platelet-derived growth factor receptor α (PDGFRA) has been associated with the progression of hepatocellular carcinoma (HCC), although the exact underlying mechanism of PDGFRA still remains unclear.
Methods: Clinicopathological value of PDGFRA was assessed in a Caucasian cohort of 136 HCC patients (resection or explant specimens) with different underlying aetiologies; the predictive value was evaluated in an independent series of 46 pre-operative needle biopsies with a follow-up of 5 years. Activation or inhibition of PDGFRA (using recombinant PDGFA/B or Crenolanib respectively) and alteration of downstream target laminin B1 (LAMB1) and its receptor integrin B1 (ITGB1) (by laminin-1 coating, shRNAs or targeted antibodies) was performed in the SNU423 and HepG2 HCC cell lines. Functional readout was done using invasion and invadopodia assays, and mouse xenograft models.
Results: In the cohort of surgical specimens PDGFRA expression was found in 66% of the samples and was significantly correlated with metastasis ($p < 0.001$), microvascular invasion ($p < 0.001$) and increased tumour size ($p < 0.05$), and was especially found in the invasive front. In the cohort of needle biopsies, PDGFRA was significantly linked with poor overall survival ($p < 0.05$). In vitro activation of PDGFRA enhanced LAMB1 expression through the LA/SSB cascade and promoted invasion and invadopodia formation, whereas inhibition of PDGFRA by Crenolanib had the opposite effect. Antibodies targeting laminin-1 or ITGB1 receptor reduced the invasive potential and altered the expression of genes involved in cytoskeleton remodelling (e.g. KRT19, ANXA3). Similar results were obtained by inhibiting Rho-associated, coiled-coil containing protein kinase 2. Moreover, knockdown of LAMB1 diminished the metastatic potential in vivo.
Conclusions: PDGFRA-LAMB1 pathway plays an important role in the invasion of HCCs and is a promising therapeutic target.

P0276
HIF2-ALPHA NEDDYLATION AS A SELECTIVE SERPINB3-DEPENDENT MECHANISM LEADING TO ITS INCREASE
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Background and Aims: SerpinB3 is a cysteine-proteases inhibitor overexpressed in hepatocellular carcinoma (HCC). In previous studies SerpinB3 has been reported to be up-regulated by hypoxia through a redox- and HIF2α-dependent mechanism. Our preliminary results suggested to act as a paracrine mediator able to induce stabilization and increased nuclear translocation of HIF2α and up-regulation of HIF2α-related genes in liver cancer cells such as CXCR4, c-Myc and CyclinD1. The ubiquitin-like molecule, NEDD8, is a key regulator of cell growth, viability and malignant transformation. Neddylation promotes timely stabilization of proteins with essential regulatory roles in an extensive variety of biological processes. It has been reported that the overexpression of global neddylation is associated with clinical HCC and it's poorest prognosis. In the present study we have investigated selective HIF2α neddylation stabilization in a serpinB3 dependent mechanism providing the advantage that this regulation has in liver cancer.
Methods: The cross-talk between SerpinB3 and HIF2α has been investigated by taking advantage of morphological, molecular and cell biology techniques in the following experimental models: (i) control HepG2 exposed to recombinant SerpinB3 (rSerpinB3); (ii) HepG2 stably transfected to overexpress SerpinB3 (HepG2/SB3); (iii) transgenic mice overexpressing SerpinB3 in the liver.
Results: Immunohistochemistry performed on liver sections from SerpinB3 transgenic mice show an impressive level of nuclear staining for HIF2α and exposure of HepG2 to rSerpinB3 results in increased nuclear levels of HIF2α. Different experimental approaches (normoxic conditions) revealed that in relation to the action of SerpinB3: (i) increased protein levels of HIF2α are unrelated to its increased transcription; (ii) increased HIF2α protein levels are unrelated to an inhibition of overall proteasome activity by SerpinB3; (iii) overexpression of SerpinB3 lead to an increase in the level of neddylated proteins and of levels of NEDD8 activating enzyme 1 (NAE-1); (iv) the use of a specific inhibitor of NAE-1 selectively reduces HIF2α levels.
Conclusions: SerpinB3 can affect the behaviour of target cells by increasing protein levels of HIF2α, followed by its nuclear translocation and induced transcription of target genes, by inducing HIF2α selective neddylation and consequent stabilization which is independent on hypoxia.

P0277

ENHANCED AND EFFECTIVE DELIVERY OF MICRORNA BY USING LIGAND MODIFIED SELF ASSEMBLED CATIONIC DIPEPTIDE NANOPARTICLES IN HEPATOCELLULAR CARCINOMA (HCC)

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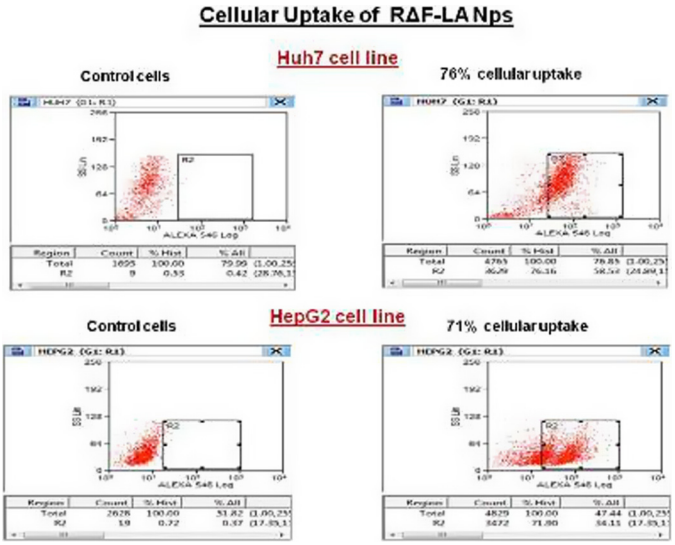
Background and Aims: HCC is a primary liver tumor with high mortality. Systemic and locoregional chemotherapy have limited role due to toxicity and low uptake of chemotherapeutic agents in HCC. RNA interference holds potential as a new therapeutic approach. However, safe and efficient nanovectors for delivery of microRNA (miRNA) are few. We aimed to create a novel vehicle to restore dysregulated gene expression in liver tumor cells by miR-199a-3p delivery. Self-assembled arginine dehydrophenylalanine-lactobionic acid nanoparticles (RΔF-LA Nps) are biodegradable, stable to proteases, non-toxic and tunable structures for targeted delivery. For selective targeting of Nps to tumor cells, we introduced ligand (LA) that can actively interact with the asialoglycoprotein receptors overexpressed on liver tumor cell surface.
Methods: Dipeptide (RΔF) was synthesized by solution phase method and LA conjugated RΔF Nps were prepared, then complexed with miRNA by electrostatic interactions. Biophysical characterizations were done by particle size analysis, zeta potential, transmission electron microscopy, electrophoretic mobility measurements, and florescence microscopy. Therapeutic efficacy was evaluated in liver tumor cell lines like HepG2 and Huh7 using MTT assay, flow cytometry, RT-PCR, wound healing/cell migration assay.
Results: We observed that the RΔF-LA Nps were spherical in morphology with mean size of 320±40 nm, zeta potential of +0.91mV±0.3mV, stable in serum, RNAse and had miRNA encapsulation efficiency of 70±4.3%. The RΔF-LA Nps were non-cytotoxic with cellular uptake of 76±2.5% in Huh7 and 71±1.7% in HepG2 cell line, significantly higher as compared to RΔF Nps. RΔF-LA Nps efficiently entered and delivered miR-199a-3p in Huh7 cell line with 10 fold increase in mature miR-199a-3p expression and a 35% decrease in CMET ($p < 0.03$), a target of miR-199a-3p. MTT assay revealed that selective reduction in Huh7 cell proliferation was 41% ($P < 0.02$). Over expression of miR-199a-3p in tumor cell lines resulted in decrease of 42±1.8% gap closure in cell migration was measured by ImageJ software and increase in apoptotic level detected by Annexin V and propidium iodide based flow cytometry analysis.



Conclusions: In this study, RΔF-LA Nps were developed and showed high affinity towards liver cancer cell lines. In vitro studies demonstrated the successful delivery of miRNA, resulted in significant inhibition in cell proliferation and migration. RΔF-LA Nps showed great promise as a selective anticancer agent against HCC.

P0278

HEPATIC STEATOSIS AND HIGH CIRCULATING FREE FATTY ACID LEVELS PROMOTE GROWTH AND INVASIVENESS OF HEPATOCELLULAR CARCINOMA

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Background and Aims: The metabolic syndrome and hepatic steatosis are independent risk factors for HCC development and progression but the underlying mechanisms are incompletely understood. Here, we aimed to investigate the influence of high free fatty acid levels and hepatic steatosis on HCC progression.
Methods: Murine HCC cells were implanted into the liver of control mice or mice fed with a high-fat diet (HFD) causing significant hepatic steatosis. Subsequently, feeding was continued with control diet or HFD for 14 days. Furthermore, different human HCC-cell lines (Hep3B, PLC, Huh7) were incubated with conditioned medium (CM) of steatotic primary human hepatocytes (PHH) or different doses of free fatty acids (FFA).
Results: Tumors grown in steatotic livers were significantly larger, showed increased mitotic activity, revealed a more invasive growth and higher expression of matrix metalloproteases (MMPs) compared to tumors formed in normal liver tissue. Under the feeding conditions used, the HFD did not cause activation of hepatic stellate cells (HSC) in the non-tumorous liver parenchyma. However, there was a significant increase of HSC activation in tumors grown in steatotic livers compared to tumors of the control group as shown by α-SMA mRNA and immunohistochemical analysis. This is critical because we and others have shown before that activated HSC promote tumorigenicity of HCC cells. Furthermore, HCC cells incubated with CM of steatotic hepatocytes caused a significant increase of proliferation as compared to HCC cells treated with CM from control PHHs. Moreover, we addition of FFA to the cell culture medium of HCC cells caused a dose dependent induction of cellular lipid accumulation, which was in line with observation that also in vivo HCCs grown in mice fed with the HFD revealed higher triglyceride levels compared to HCCs in control livers. Noteworthy, steatotic HCC cells showed enhanced migratory activity in Boyden Chamber assays and higher expression of MMPs compared to HCC control cells.
Conclusions: Different mechanisms promote HCC growth in steatotic livers. Once, lipid accumulation in hepatocytes causes secretion of factors promoting tumor growth. Furthermore, desmoplastic reaction is enhanced in HCC in steatotic tissues and systemic metabolic changes associated with the metabolic syndrome such as hyperlipidemia seem to directly affect HCC cells offering a further option for HCC prevention and treatment.

P0279

SLC25A11 PROMOTES CELL PROLIFERATION IN HEPATOCELLULAR CARCINOMA SPHEROIDS AND ORTHOTOPIC TUMOR GROWTH

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Background and Aims: Hepatocellular carcinoma (HCC) is the most common form of liver cancer and a leading cause of cancer-related deaths in the world. Cancer cells exhibit increased mitochondrial cholesterol (mChol), which contributes to apoptosis and chemotherapy resistance by impairing mitochondrial outer membrane permeabilization. However, hepatocellular mChol accumulation promotes steatohepatitis due to mitochondrial GSH (mGSH) depletion determined by defective GSH import by the 2-oxoglutarate carrier (SLC25A11). Hence, the purpose of our study was to characterize the status of mGSH in HCC and to explore the role of SLC2511 in HCC spheroid formation and i*n vivo* liver tumorigenesis.
Methods: Hep3B cells were cultured in monolayers or in 3D spheroids using PolyHEMA-coated plates with or without SLC25A11 silencing by siRNA. Hep3B monolayers were subject to hypoxia ($1\%O_2$) to examine the impact on reactive oxygen species (ROS) and cell death. Spheroids growth and size was monitored during 6 days with or without GSH ethyl ester (GSHee). Immunohistochemical analyses of PCNA, KI67 and pimonidazole (PIMO) were performed in collected spheroids at day 7. Tumor initiating stem cells (TICs) were stably transfected with two silencing vectors targeting SCL25A11 and injected in the parenchyma of the left liver lobe of immunodeficient mice (NOG mice). Animals were sacrificed three weeks later and tumor growth was analyzed in all animals.
Results: SLC25A11 silencing in Hep3B monolayers depleted mGSH by 50–60%, resulting in hypoxia-induced ROS overgeneration (2 fold) and sensitization to hypoxia-induced cell death (70%). Moreover, Hep3B spheroids growth was reduced upon SLC25A11 silencing by 50–60% compared to control spheroids. The effect of SLC25A11 silencing on spheroids growth was reversed by mGSH replenishment with GSHee treatment, while GSHee *per se* increased size in control spheroids. Core spheroids cells stained with PIMO and Ki-67 and PCNA positive cells were reduced in siRNA SLC25A11-treated spheroids compared to siRNA scrambed-treated spheroids. SCL25A11 silencing in TICs also caused reduced cell number and proliferation without effect in cell size. Moreover, in vivo tumor size and multiplicity were reduced in mice injected with stably genetic SLC25A11 silenced TICs.
Conclusions: SLC24A11 silencing in Hep3B-3D spheroids and TICs orthotopic tumor model reduced tumor growth *in vitro* and *in vivo*, suggesting that targeting this mitochondrial carrier may be of potential relevance in HCC.

P0280

EFFECT OF EXTRACELLULAR MATRIX ON CANCER STEM CELLS

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Background and Aims: Activated hepatic stellate cells are responsible for the excessive deposition of extracellular matrix (ECM) in chronically damaged livers. ECM proteins as well as their proteolytic fragments have been implicated in playing a crucial role in tumor growth, metastasis, and tumor neo-angiogenesis. Whereas some of the molecules in the tumor microenvironment have an effect on vasculature, others have a direct effect on the tumor cells, altering their behavior and phenotypic properties. An interesting feature is the increasing evidence that the ECM is an essential component of the stem cell niche and that it can directly regulate stem cell differentiation. However, the molecular details of how this is achieved have only just started to emerge. Our aim is to investigate the direct effect of ECM on hepatocellular carcinoma (HCC), mainly focusing on cancer stem cell (CSC) properties, migration and proliferation.
Methods: Human immortalized hepatic stellate cells (LX2) were stimulated with TGF-beta and/or the fibrin degradation product Fragment E (FnE) for 48 hrs. Medium from these cells was collected and used for subsequent stimulation of the human hepatoma cell line HepG2. HepG2 cells were grown on plates coated with ECM proteins, including collagen, fibrinogen, fibrin, FnE and fibrinogen-like-protein 1. Cells were harvested after 48 hrs and RNA was isolated for subsequent qPCR analysis on CSC markers. Scratch wound assays were performed to assess migration. Inhibitors of collagen-synthesis and integrin-signaling were used to study the involvement of collagen.
Results: FnE and TGFβ lead to a 2-fold increased expression of smooth muscle actin and collagen in HSC, compared to TGFβ alone. Stimulating HepG2 cells with medium from these HSC caused a significant increase of CSC-markers, and increased migration in the condition where TGFβ and FnE were used together to stimulate the HSC. This effect was diminished when a collagen-synthesis inhibitor was added to the HSC during stimulation with TGFβ and FnE, suggesting that perhaps the increased collagen production by activated HSC could be a key influence on tumor behavior. Growing HCC cells on collagen-coated plates also increased expression of CSC-markers prominin-1 and CK19. Other ECM proteins did not have this effect.
Conclusions: Fibrin fragment E can stimulate HSC to secrete factors which cause cancer cells to de-differentiate towards a more CSC-phenotype, associated with more aggressive tumours.

P0281

DECELLULARISED HUMAN LIVER AS A NATURAL SCAFFOLD FOR 3D-DISEASE MODELLING

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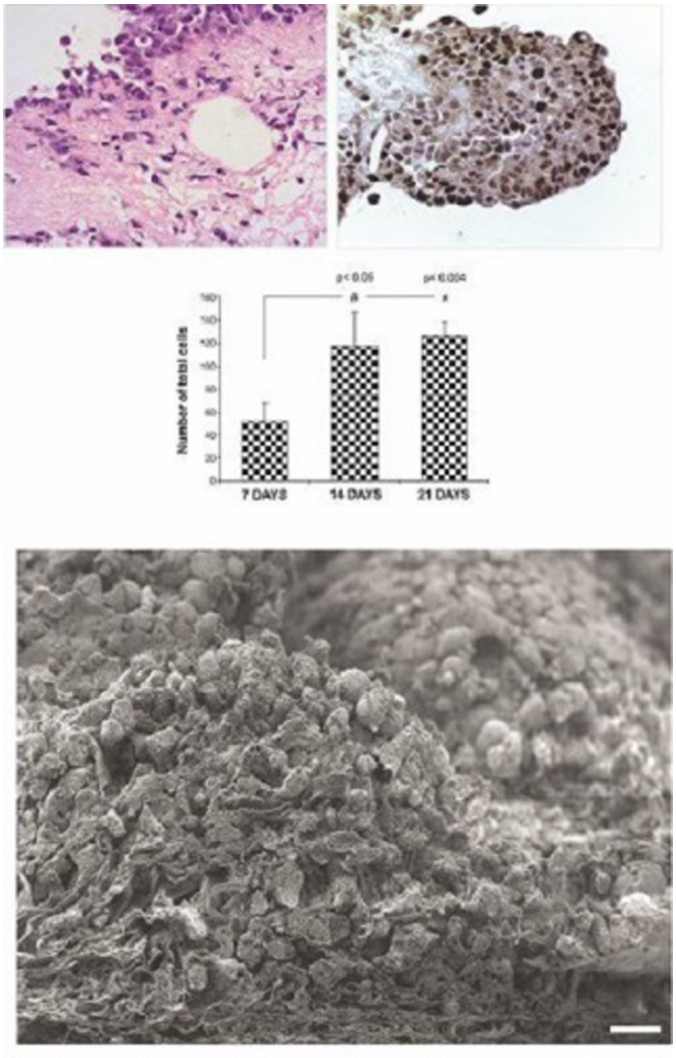
Background and Aims: It is generally well recognised that animal models are poor surrogates for understanding the pathophysiology of liver disease, and generally unrepresentative when it comes to studying liver fibrosis, cancer and drug metabolism. The ideal

model would use human liver, or a biologically relevant 3D in vitro model of human liver cells grown on an extracellular matrix (ECM) derived from healthy liver tissue.
The aim of this study was to develop a rapid protocol for the decellularisation of small samples of human liver and demonstrate repopulation with cultured human liver cell lines, namely hepatocarcinoma (HepG2), metastatic adenocarcinoma (SK-Hep-1) and hepatic stellate (LX2) cells.
Methods: Liver tissue cubes (5×5×5 mm, i.e. 0.125 cm^3) were dissected from human livers unsuitable for transplantation. The decellularisation of the human liver cubes was completed within 3 hours of incubation and agitation in our decellularization medium (1. deionized water, 2. detergents and 3. enzymes [trypsin]), and following optimization of pre-existing protocols for animal tissues. The decellularization efficiency was determined by immunohistochemistry for ECM components and residual DNA, scanning electron microscopy, as well as DNA and ECM protein quantification. The liver cube scaffolds were seeded and repopulated by HepG2, SK-Hep-1 and LX2 for up to 21 days.



Results: This innovative protocol resulted in liver cube scaffolds with a preserved 3D structure and ECM composition, while DNA and cellular residues were successfully removed. Human liver scaffolds were progressively repopulated for up to 21 days with LX2, SK-Hep-1 and HepG2 cells which showed remarkable viability, motility and proliferation associated with remodelling effects on the surrounding ECM. Notably, the expression of some genes and proteins involved in liver fibrosis and cancer was different between the 2D and the 3D system.
Conclusions: This is the first report describing an efficient protocol to completely decellularize human liver cubes. The decellularization protocol was rapid and maintained the natural 3D structure and ECM composition and organisation. This is a key advance in the development of 3D technologies for the study of human liver fibrosis and cancer.

P0282

THE LONG NON CODING RNA UC.158 MODULATES GROWTH OF Wnt/β;-CATENIN DRIVEN HEPATOCELLULAR CARCINOMA (HCC)

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Background and Aims: Transcribed-Ultraconserved-Regions (T-UCR) are long ncRNAs which are conserved across the species and are involved in carcinogenesis. Here we look at T-UCRs downstream of Wnt/β-catenin pathway in HCC.
Methods: $APC^{fl/fl}$ mice developed Wnt/β-catenin dependent hepatocarcinoma (HCC). Expression of T-UCR was assessed by microarray, qtPCR, and In Situ Hybridization. Mutations of CTNNB1-exon3 were analysed by direct-sequencing. Cell viability was assessed by Alamar Blue, apoptosis by Caspase3/7GLO.
Results: We profiled T-UCR expression in the liver of $APC^{fl/fl}$ and $APC^{+/+}$ mice. Over-expression of uc.158− could differentiate Wnt/β-catenin dependent HCC from normal liver and from β-catenin negative DEN-induced HCC. RACE showed uc.158− is transcribed as an independent antisense ncRNA. uc.158− was over-expressed in human HepG2 vs Huh7 cells and in Hepatic Stem Cells vs normal hepatocytes, in line with the activation of the Wnt pathway. In vitro modulation of β-catenin resulted in alteration of uc.158− expression in human HCC cell lines. uc.158− expression was increased in CTNNB1-mutated compared to non-mutated human HCCs, and in human HCC with nuclear localization of β-catenin. LNA-mediated inhibition of uc.158− reduced anchorage cell growth by 60% (probe1) and 70% (probe3) at 72 hrs in HepG2 cells. HepG2 3D-spheroid formation was reduced to 34% and 40% after silencing of uc.158− with probe 1 and 3 respectively. Inhibition of uc.158− also reduced spheroid-based cell migration. FACS analysis showed no changes in the phases of cell cycle, but for an increased number of cells in the subG0-G1 phase after uc.158 silencing. Inhibition of uc.158 increased cleavage of caspase3/7, but did not affect cytotoxicity. Computational analyses identified binding sites (ddG score <10) for 14 miRNAs (conserved across species) within the uc.158− miRNA profile in the $APC^{fl/fl}$ and $APC^{+/+}$ mice showed 10 of these miRNAs being down-regulated in HCC compared to controls, suggesting that uc.158− may act as a competing endogenous RNA. Amongst these, miR-193b, miR-346 and miR-615 were increased after silencing of uc.158− in HepG2 cells. miR-193b expression was reduced in HepG2 compared to Huh7. Enforced expression of uc.158− in Huh-7 cells reduced expression of miR-

193b. Data from literature confirm reduced expression of miR-193b in human HCC.
Conclusions: We showed that uc.158− is activated by Wnt/β-catenin pathway in HCC and drives HCC growth. Thus it may represent a promising target for the development of novel therapeutics.

P0283
ADENOSINE MONOPHOSPHATE ACTIVATED KINASE (AMPK) IN CANCER AND HUMAN HEPATIC STELLATE CELL CROSSTALK
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Background and Aims: Human hepatic stellate cells (HSC) are important in fibrosis associated with hepatocellular carcinoma (HCC) progression. In addition, it has been demonstrated that HCC modifies HSC behaviour. Hence, tumour-stromal interactions are considered as a potential target for anti-cancer and anti-fibrogenic therapy. Adenosine monophosphate-activated kinase (AMPK) and liver kinase B1 (LKB1) have a critical function in energy homeostasis regulation in HCC. However, little is known about the effects of AMPK-related HCC metabolic alterations on the microenvironment, particularly activated HSC. In this study, we utilized primary HSC and HCC cell lines to systematically analyse the role AMPK/LKB1 on tumour-stromal crosstalk in vitro.
Methods: Conditioned media (CM) of HepG2 and PLC/PRF/5 HCC cells were harvested after 48 hrs of incubation in serum-free medium. Primary HSC were treated with CM from either cell lines for 24 hrs. Measured endpoints included proliferation (BrdU incorporation), metabolic activity (MTS assay), and gene and protein expression. To assess the role of AMPK, the above responses at 24 hrs were evaluated in presence of AICAR (1mM), an AMPK agonist.
Results: Conditioned media from HepG2 and PLC/PRF/5 had differential effects on HSCs proliferation and AMPK activation status. While HepG2-CM stimulated HSC proliferation and induced AMPK-Ser485-phosphorylation, PLC/PRF/5 CM had no effect on HSCs proliferation while inducing AMPKThr172/LKB1 phosphorylation. These experiments suggest that the AMPK phosphorylation status in HSCs correlates with their proliferation rates and the presence of a soluble "factor" in the supernatant of HCC cells able to induce significant effects on hHSC proliferation. Differential size-fractionation centrifugation identified a factor of <10kDa with inhibitory effects and a factor of >30kDa that stimulates HSC proliferation. The effect of both HCC conditioned media on HSC proliferation was prevented by the AMPK agonist AICAR, which induced the phosphorylation of AMPK-Thr172 and LKB1. Lastly, HepG2- and PLC/PRF/5-CM modified HSC gene expression of several profibrogenic and proinflammatory genes. These effects were further altered in the presence of AICAR.
Conclusions: Our results suggest a complex crosstalk between HCC and HSC during liver cancer development. Understanding the dual mechanisms mediated by multisite phosphorylations by which AMPK is regulated could allow identifying new targets to inhibit both cancer development and the accompanying stromal reaction.

P0284
TYPE 2 DIABETES PROMOTES HEPATOCARCINOGENESIS BY INHIBITING THE INDUCTION OF SENESCENCE AFTER DNA DAMAGE
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Background and Aims: Diabetes and cancer are both serious and growing health problems worldwide. A number of clinical studies have demonstrated a positive correlation between type 2 diabetes and an increased cancer risk including hepatocellular carcinoma (HCC). The mechanism by which type 2 diabetes promotes hepatocarcinogenesis is largely unknown. FoxO3, a Forkhead-box transcription factor family member, is known to be a master regulator in the insulin signalling pathway and plays a critical role in glucose metabolism. Activation of FoxO3 leads to glucose production and resistance to insulin in hepatocytes. The aim of our study was to investigate the underlying mechanism of type 2 diabetes-enhanced tumorigenesis.
Methods: In order to test this hypothesis, we investigated a mouse model expressing a constitutively active form of FoxO3 (FoxO3CA) in mouse hepatocytes, which show hyperglycemia, hyperinsulinemia, impaired glucose tolerance and insulin resistance. Potential signalling mechanisms in the frame of type 2 diabetes-associated tumorigenesis were investigated using several human HCC cell lines, such as HepG2 and HuH7.
Results: Activation of hepatic FoxO3 signalling led not only to insulin resistance and type 2 diabetic characteristics, but we also observed histomorphological alterations, such as the induction of hepatic dysplasia. Hepatocytes expressing FoxO3CA were found to become relatively atrophic, exhibiting a histological similarity to so-called small cell dysplasia or small cell changes (SCC) which have been observed in livers from patients of pre-cancerous stage. Moreover, we observed intensively hypertrophic hepatocytes (Large cell changes = LCC), which obviously lost FoxO3 expression and apparently showed an activation of the p53-p21 senescence pathway. In contrast, in SCC areas severe DNA damage and expression of several HCC markers could be detected, which could not be seen in LCC. Furthermore, we observed that status of FoxO3 activity and p21 expression in HepG2 and HuH7 cells was consistent to what we observed in the mouse model.
Conclusions: Hepatic FoxO3 over-expression mimicking type 2 diabetes seems to promote hepatocarcinogenesis, presumably due to the inhibition of senescence signalling after DNA damage. These findings are of major relevance with regards to type 2 diabetes-induced cancers.

P0285
INACTIVATION OF CYCLIN E1 ACTS PRO-APOPTOTIC AND ANTI-PROLIFERATIVE IN PRIMARY HEPATOMA CELLS AND PROTECTS FROM HEPATOCARCINOGENESIS IN MICE
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Background and Aims: E-type cyclins (CcnE1, CcnE2) are regulatory subunits of Cyclin-dependent kinase 2 (Cdk2) and control the

transition of quiescent cells into the cell cycle. Surprisingly, single genetic deletion of CcnE1, CcnE2 or Cdk2 does not inhibit cell proliferation during embryonic development or liver regeneration. In the present study we evaluated if E-type cyclins may have a specific role for hepatocarcinogenesis.
Methods: We used mice with constitutive deletion of Cyclin E1 (CcnE1$^{-/-}$) or Cyclin E2 (CcnE2$^{-/-}$) and conditional knockout mice with hepatocyte-specific inactivation of Cdk2 (Cdk2$^{\Delta hepa}$), respectively. To induce hepatocellular carcinoma (HCC), mice were treated with a single dose of Diethylnitrosamine (DEN) at the age of two weeks and analysed for tumor initiation and progression after 24 and 40 weeks.
Results: 24 weeks after DEN treatment 40% of wildtyp (WT) and CcnE2$^{-/-}$ mice developed few small tumor nodules, whereas 100% of CcnE1$^{-/-}$ mice revealed normal liver morphology lacking visible tumor nodules. At the age of 40 weeks, 100% of DEN-treated WT and CcnE2$^{-/-}$ mice developed HCC. In sharp contrast, HCC incidence in CcnE1$^{-/-}$ animals was only 50%, and the overall number of tumor nodules was tenfold reduced in comparison to WT and CcnE2$^{-/-}$ mice. Ablation of CcnE1 also protected from HCC in transgenic mice with hepatic over-expression of the proto-oncogene c-*myc*. Interestingly, Cdk2$^{\Delta hepa}$ mice and Cdk2$^{\Delta hepa}$CcnE2$^{-/-}$ double mutants were also strongly protected from DEN-mediated hepatocarcinogenesis. We next isolated primary, CcnE1-floxed hepatoma cells from DEN-treated CcnE1$^{f/f}$ mice and inactivated CcnE1 *in vitro* using EGFP-labeled, cre-expressing adenoviruses. Confocal live cell imaging revealed that acute CcnE1 inhibition in transformed hepatoma cells effectively results in apoptosis within 72 hours. In addition, CcnE1-depleted hepatoma cells showed strong activation of caspase-3, and de-regulation of S-phase and cytokinesis related genes and proteins, respectively.
Conclusions: Our data demonstrate that CcnE1 – but not its homologue CcnE2 – is essential for proliferation and survival of immortalized hepatoma cells *in vivo* and *in vitro*. This requirement of CcnE1 seems to be dependent on the availability of Cdk2. We hypothesize that hepatoma cells get addicted to high CcnE1 expression levels during the transformation process. Thus, acute inhibition of CcnE1 during HCC progression could be a novel therapeutic option.

P0286
C-MET SIGNALING CONTRIBUTES TO CARCINOGENESIS OF CHOLANGIOCARCINOMA AND IS BLOCKED BY LY2801653, A SMALL MOLECULE INHIBITOR

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Background and Aims: The incidence and mortality rate of cholangiocarcinomas (CCC) are increasing worldwide and they represent the second most common primary hepatobiliary cancer. Currently, the combination of Gemcitabine and Cisplatin is the standard chemotherapeutic regimen for patients undergoing first line treatment. Further improvements in the treatment of CCC, especially targeted therapies are still needed to overcome this deadly disease. c-MET signaling is important for a variety of normal cellular function, but aberrant expression plays also a major role in carcinogenesis in many human cancer diseases. The role of c-Met in the carcinogenesis of CCC remains still unclear. In this study we investigated the effects of LY2801653, a small-molecule inhibitor with potent activity against MET kinase, in human CCC cell lines (TFK-1, SZ-1) and *in vivo* using a Xenograft mouse model.
Methods: We analyzed the effect of LY2801653 by testing different dosages on CCC growth and epithelial plasticity by using WST-1 assay, wound healing assay, invasion assay, colony formation assay, apoptosis assay, senenscence assay, Western Blot, Immunohistochemistry and in vivo CCC tumor growth by using an intra- and extrahepatic Xenograft model.
Results: c-MET was expressed in 72% of analyzed intra- and extrahepatic human CCC tissues and in TFK-1, SZ-1 cell lines. MET inhibition was achieved by blocking phosphorylation of MET with LY2801653 and subsequent down regulation of downstream targets like p-Erk, p-AKT and RAC1 which are associated with important cell regulatory functions. LY2801653 treatment mainly induced senescence and partially apoptosis as the key mechanisms for cell death. Treatment showed in Xenograft models potent anti-tumor activity with a decrease of cell proliferation and increased cleavage of PARP and expression of P21.
Conclusions: Our data demonstrates that c-MET signaling is important for CCC carcinogenesis and that LY2801653 treatment is a potential, effective alternative strategy to treat CCC.

P0287
LIVER-SPECIFIC EXPRESSION OF HBs-Ag LEADS TO A SHIFT OF TUMOR DIFFERENTIATION IN Trp53-DELETED LIVER TUMORS

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Background and Aims: Around 50% of all human hepatocellular carcinomas are associated with a chronic HBV infection. Furthermore, up to 70% of HCC are characterized by defects in the p53-signaling pathway. The loss of p53 is often characterized with a mixed tumor differentiation and a stem cell gene expression pattern which is associated with a poor prognosis. It is unknown how HBV infection and p53 loss affects tumor differentiation and gene expression profiles.
Methods: For the induction of liver tumors, we crossed a conditional Trp53 mouse with an Alb-HBs mouse (HBs-Ag under the albumin-promoter) and a liver-specific AlfpCRE mouse (CRE expression under the albumin-alphafetoprotein-enhancer-promoter) to induce a liver-specific deletion of the Trp53 gene during embryogenesis and a chronic liver damage by the HBs-Ag. Moreover, we compared these mice with single Trp53 deleted mice without HBs-Ag expression.
Results: Tumor incidence in mice with liver-specific Trp53 deletion was significant lower (32.4%) compared to mice with Trp53 deletion and chronic liver damage (100%) at an age of 12–14 months. In mice without chronic liver damage, 79.7% of the tumors showed mixed differentiation with hepatocellular carcinoma as well as cholangiocellular carcinoma (HCC-CC), and 10.9% showed only hepatocellular carcinoma (HCC). This mixed tumor differentiation decreased to 30% HCC-CC in mice with chronic liver damage by HBs-Ag. The expression of HBs-Ag results in a shift of tumor differentiation towards HCC (70%). Tumors with a mixed differentiation exhibit an increased expression of stem cell markers (CD90, CD133, Sox9).
Conclusions: Our study shows that Trp53 deletion alone leads to liver tumor formation with a mixed differentiation with stem cell features. The addition of a chronic liver damage by HBs-Ag leads to a partial shift towards hepatocellular carcinoma.

P0288

CXCR4 INHIBITION REVERTS IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT AND FACILITATES ANTI-PD-1 IMMUNOTHERAPY IN SORAFENIB-TREATED HEPATOCELLULAR CARCINOMA

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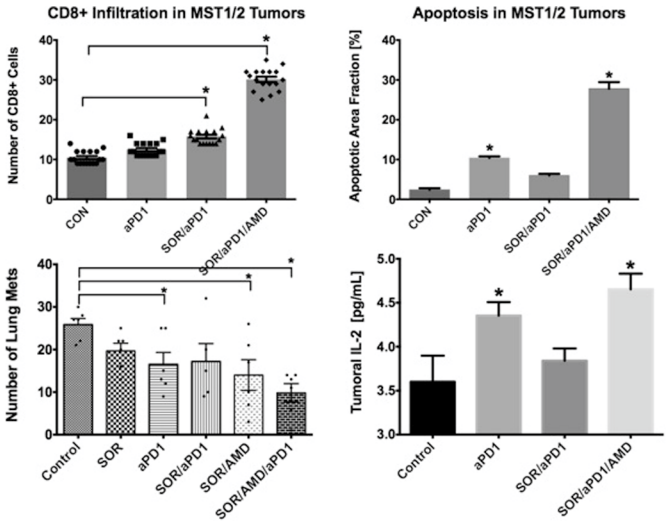
Background and Aims: Sorafenib – a broad tyrosine kinase inhibitor – is the only approved systemic therapy for advanced hepatocellular carcinoma (HCC), but provides limited survival benefits. We have shown in mouse models that after sorafenib treatment, intratumoral hypoxia is increased and may fuel evasive resistance.
Methods: Two sophisticated HCC models with and without underlying cirrhosis were used: the orhtotocpic HCA1 tumor implantation in C3H mice and the GEM model of YAP1-driven HCC in MST1/2 knockout mice. Fibrosis was induced by iterative CCl_4 administration. The growth of intrahepatic HCCs was evaluated by high-frequency ultrasound imaging. Treatment regimens were started when tumor size reached 3×3 mm (14 mm^3): Sorafenib (50 mg/kg/day gavage), CXCR4 inhibitor AMD3100 (10 mg/kg, s.c. by osmotic minipump), antibody against murine PD-1 (100 μg every 3 days, i.p.).



Results: Using orthotopic HCC models, we now show that increased hypoxia after sorafenib treatment induces an immunosuppressive tumor microenvironment characterized by increased intratumoral expression of the immune checkpoint inhibitor programmed death-ligand 1 (PD-L1) and accumulation of T-regulatory cells and M2-type macrophages. The recruitment of these cells was mediated by hypoxia-induced upregulation of stromal cell-derived 1 alpha (SDF1α). Inhibition of the SDF1α receptor (C-X-C receptor type 4/CXCR4) using AMD3100 prevented the immunosuppressive reprogramming of the microenvironment after sorafenib treatment, inhibited tumor growth, reduced lung metastasis, and improved survival. However, combination of AMD3100 and sorafenib did not increase cytotoxic CD8+ T-lymphocyte infiltration. Moreover, *in vivo* antibody blockade of the PD-L1 receptor PD-1 showed anti-tumor effects in treatment-naïve tumors, and additional anti-tumor activity when combined with sorafenib and AMD3100, but not when combined with sorafenib only.
Conclusions: Anti-PD-1 treatment seems to boost immune response in HCC tumors. However, when used with sorafenib – concomitant targeting of the hypoxic and immunosuppressive microenvironment with agents such as CXCR4 inhibitors may be required.

P0289

EVOLUTION ANALYSIS BY DEEP SEQUENCING REVEALED HIGH IMPACT OF THE MITOCHONDRIAL GENOME IN HEPATOCELLULAR CARCINOMA INITIATION AND PROGRESSION

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Background and Aims: Hepatocellular carcinoma (HCC) is highly malignant tumor, prone to multicentric occurrence (nodule-in-nodule pattern) and triggered by a wide variety of genetic alterations. Herein, we aimed to study the occurrence of disease related mitochondrial mutations variants by a comprehensive ultra-deep sequencing approach as a novel tool for hepatocellular cancer follow-up and prognosis.
Methods: Macrodissected 48 HCC nodules, representing different tumor grades, and adjacent non-cancerous tissues from ten patients were investigated. One hundred-eight primer sets spanning the whole mtDNA were designed and automatic single PCR set-up was established using the Biomek robotic system. Target enriched libraries of mtDNA was sequenced by means of the MiSeq platform and data analysis was performed by the CLC Cancer Research Workbench.
Results: The mitochondrial (mt) genome is highly susceptible to DNA alterations due to the lack of protective histones and a limited repair system. Here, we are using mt-variations as a way of focal cancer barcoding and tracking. Whole mt-genome analysis revealed high frequency of variants in the D-Loop region and the MT-CYB gene. In particular, in HCC nodules of non-cirrhotic origin a wide spectrum of mt-variants with high frequency were identified. However, highly frequent mt-variants occur not only in HCC nodules but also in peri-tumorous parenchymal foci raising the possibility that mtDNA mutations might precede nuclear alterations and play a role in the pre-tumor machinery. Furthermore, the occurrence of identical mt-variants in different nodules of one liver sample indicates the monoclonal HCC origin. Most notably, the increasing numbers of mt-variants as well as the increasing frequency of a particular mt-hot-spot mutation refer to the progression of the HCC dedifferentiation.
Conclusions: In conclusion, screening of mitochondrial genome by ultra-deep sequencing is a reliable and useful molecular tool to identify pre-tumorous nodules with high transformation potential and to illustrate tumor clonality and history. Early appearance and high frequency of mt-hot spot mutations associated with HCC development and nuclear genomic alterations provide novel insights in cancer diagnostics and therapeutic approaches.

P0290

PLACENTAL GROWTH FACTOR INHIBITION MODULATES THE INTERPLAY BETWEEN HYPOXIA AND THE UNFOLDED PROTEIN RESPONSE IN HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality worldwide. We previously showed that inhibition of Placental growth factor (PlGF) exerts antitumour effects and induces vessel normalization in HCC, possibly reducing hypoxia. However, the exact mechanism of PlGF inhibition in HCC remains unclear. Since hypoxia signalling and the unfolded protein response (UPR) have been implicated in tumour progression, we assessed the interaction between PlGF and these tumoural adaptation processes.
Methods: PlGF knockout mice and monoclonal anti-PlGF antibodies were used in a diethylnitrosamine-induced mouse model for HCC to investigate the effect of PlGF inhibition on UPR activation and tumour hypoxia. In addition, we examined the interaction between the UPR, hypoxia and PlGF expression in human HCC cell lines.
Results: Both genetic and pharmacological inhibition of PlGF significantly reduced diethylnitrosamine-induced chaperone levels ($p < 0.05$) and the activation of the PKR-like endoplasmic reticulum kinase (PERK) pathway of the UPR ($p < 0.01$). Also tumour hypoxia was attenuated by both, as shown by reduced pimonidazole staining in the tumour nodules ($p < 0.05$). Interestingly, hypoxia markedly activated the PERK pathway in human HCC cells ($p < 0.01$), suggesting PlGF inhibition may diminish PERK activation by improved oxygen delivery via vessel normalization. Finally, we found that both hypoxia and two chemical inducers of endoplasmic reticulum stress, tunicamycin and thapsigargin, increased the expression of PlGF in HCC cells ($p < 0.01$), predominantly via the inositol-requiring enzyme 1 (IRE1) pathway of the UPR.
Conclusions: These findings indicate that PlGF inhibition reduces UPR activation by tempering hypoxia in experimental HCC and that the UPR, in turn, is able to induce PlGF expression, suggesting the existence of a feedback mechanism of hypoxia-mediated UPR which stimulates PlGF-mediated angiogenesis.

P0291

THE ONCOSTATIC ACTION OF SORAFENIB ON LIVER CANCER CELLS IS CHANGED TO AN ONCOLYTIC EFFECT FOLLOWING PRE-TREATMENT WITH CHLOROQUINE: DEMONSTRATION OF SYNERGY BETWEEN CHLOROQUINE AND SORAFENIB AS A POTENTIAL NOVEL APPROACH TO TREATMENT OF HCC

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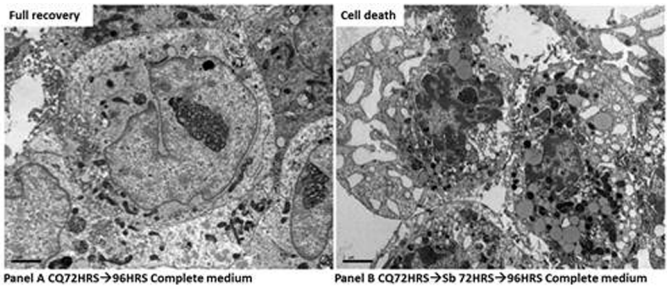
Background and Aims: Sorafenib (Sb), a multikinase inhibitor is the elective treatment for patients with advanced HCC. Currently no preventive strategies or cost-effective treatment option(s) for HCC are available. Chloriquine (CQ) has been implicated as a chemosensitizer when used in combination with anti-cancer drugs. The aim of this study is to determine if pretreatment with CQ potentiates the efficacy of Sorafenib admistered in subpharmacological doses.
Methods: Response to CQ and Sb, alone and in combination, was tested in Huh7 and HepG2 cells as follows: (i) untreated; (ii),(iii) CQ (15 μM), Sb (3 μM) 96 h treatment (IC50) followed by 96 h recovery, respectively; (iv) CQ 72 h treatment followed by Sb 24–72 h treatment and 96 h recovery. Cell response was evaluated by MTS assay, electron microscopy (EM) and cell cycle analysis (FACS analysis).
Results: CQ treatment had an oncostatic effect on HCC by causing an impaired cytoplasmic compartmentalisation resulting in G2 cell cycle arrest. Both mitochondria and nuclei preserved their structure/functions. When CQ was removed, the cells were capable to fully overcome the CQ-induced oncostasis by restoring their ultrastructure (Figure, Panel A), cell cycle, and growth rate. Low doses of Sb alone caused a transient reduction in cell growth with no ultrastructural changes. Interestingly, when Sb was added to CQ-damaged cells, their ability to recover was completely eliminated and autophagic cell death induced (Figure, Panel B).
Conclusions: CQ-treatment alone had a transient oncostatic effect on HCC cells. Nevertheless, when low doses of Sb were administered to CQ-sensitised cells, an oncolytic effect on HCC cells associated with autophagic cell death was achieved. Altogether, these data underscore the synergy between CQ and Sb as a potentially novel approach to the treatment of HCC.



P0292

KNOCKDOWN OF NANOG REVERSES THE CHEMORESISTANCE TO DOXORUBICIN IN LIVER CANCER STEM-LIKE CELLS BY REDUCING ABCB1 AND ABCG2 EXPRESSION

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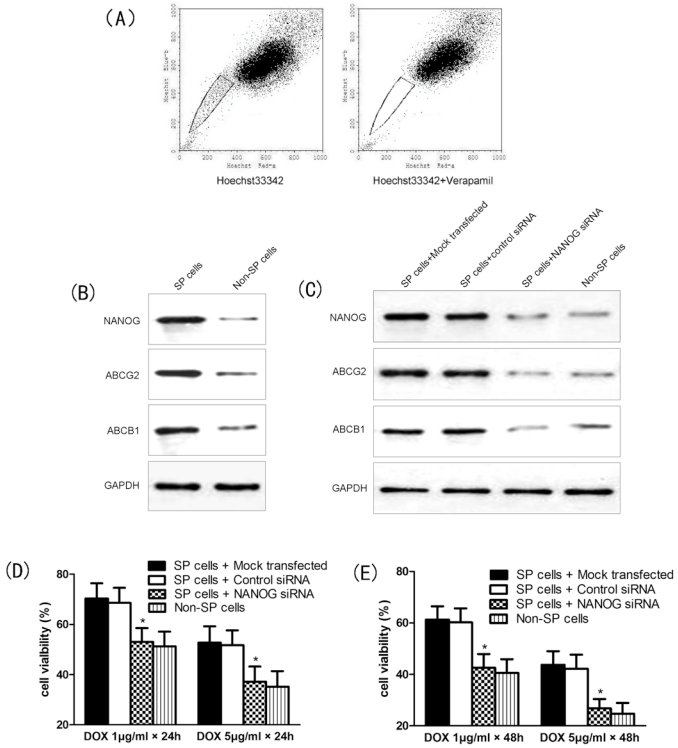
Background and Aims: Chemoresistance of cancer cells is one of the major reasons for the failure of liver cancer chemotherapy in clinic. Cancer stem cells (CSCs) are regarded as the origin of tumors, which are resistant to current chemotherapies. NANOG is one of the most important core markers of CSCs in maintaining the self-renewal and pluripotency. However, the association between NANOG and chemoresistance in CSCs remains uncertain. The aim of this study was to investigate whether NANOG inhibition sensitizes human liver CSCs to doxorubicin treatment.
Methods: Liver cancer stem like cells were purified from liver cancer cells Hep3B by side population (SP) analysis. siRNA was employed to inhibit NANOG expression in SP cells and chemosensitivity to doxorubicin as well as expression of NANOG, ABCB1 (MDR1, P-gp) and ABCG2 (BCRP1) were analyzed by CCK8 method and Western blot, respectively.
Results: SP cells were detected in liver cancer cells Hep3B (Figure A). NANOG, as well as ABCB1 and ABCG2, are overexpressed in SP cells compared with Non-SP cells (Figure B). Meanwhile, NANOG siRNAs efficiently decreased the expression of NANOG at both mRNA and protein levels (Figure C). Knockdown of NANOG resulted in enhanced chemosensitivity to doxorubicin (Figures D,E) and reduced expression of ABCB1 and ABCG2 in SP cells (Figure C).
Conclusions: Our findings suggest that knockdown of NANOG may reverse the chemoresistance of liver CSCs to doxorubicin by

reducing ABCB1 and ABCG2 expression. Thus NANOG can prove to be a novel potential therapeutic target for liver cancer treatment.



P0293

TARGETING GLUCOSYLCERAMIDE SYNTHASE INCREASES SORAFENIB EFFICACY AND EVADES SORAFENIB RESISTANCE IN HCC MODELS

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Background and Aims: Multikinase inhibitor sorafenib has limited efficacy in the treatment of advanced hepatocellular carcinoma (HCC) due to primary and acquired drug-resistance mechanisms. Ceramide participates in chemotherapeutic cytotoxicity and the induction of the ceramide-modifying enzyme glucosylceramide synthase (GCS) is common in cancer resistance.
Methods: Hepatoma cell lines (HepsG2 and Hep3B) with sorafenib sensitive or with sorafenib resistance were selected after long term exposure to sorafenib (0 to 5 μM). GCS reduction was accomplished genetically by RNA interference and pharmacologically via inhibitor administration (PDMP). Western blots in total, cytosolic and mitochondrial extracts and qPCRs were performed after sorafenib exposure. ROS production, mitochondrial membrane permeabilization, caspase activity and ATP measurements were analyzed in sorafenib-treated hepatoma cells. Subcutaneous tumors, sensitive or with sorafenib resistance, were analyzed after injection of HepG2 cells on the flanks of nude mice
Results: Ceramide metabolism is activated in hepatoma cells after short-time exposure to sorafenib, principally by increased expression of the ceramide degrading enzyme GCS. Hep3B and HepG2 cells treated with GCS inhibitor (PDMP) or transfected with siRNAs against GCS exhibit higher sorafenib-induced cell death. The mechanism of sensitization is autophagy- and caspase-independent, but caused via mitochondria after energetic collapse as detected by cytochrome c release and ATP depletion. GCS targeting, genetically and pharmacology, is also effective in resensitizing sorafenib-resistant hepatoma cells after long-term exposure (12 months) to the drug. In vivo, administration of GCS inhibitor (PDMP), in combination with sorafenib, reduced tumor growth of HepG2 cells after subcutaneous injection in nude mice, compared with sorafenib treatment alone. Moreover, GCS inhibition concomitant with sorafenib administration was also effective in reducing sorafenib-resistant tumors in mice.
Conclusions: GCS reduction increases the cytotoxic accumulation of ceramide in sorafenib-treated hepatoma cells via mitochondrial-dependent cell death and improves sorafenib efficacy in HCC models, both in sorafenib sensitive and resistant hepatoma cells. Strategies aimed to increase ceramide toxicity, particularly through GCS inhibition, may increase sorafenib effectiveness in HCC management.

P0294

ONCOGENESIS: A POTENTIAL "OFF-TARGET" EFFECT OF RADIOFREQUENCY ABLATION

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Background and Aims: Hepatocellular carcinoma (HCC) is the 2nd most common cause of worldwide cancer-related mortality. Radiofrequency ablation (RFA) treatment for HCC, facilitates thermal injury and thus cell death via coagulation necrosis to the tumor through electromagnetic energy deposition. The 5 years survival rate of patients treated with RFA remains approximately 50% with 70–81% new tumor recurrence. Our aim is determine the tumorigenic mechanism of HCC RFA.
Methods: Nine months old MDR2 KO female mice that have dysplastic nodules underwent either RFA (tip temperatures of 70±1°C for 5 min [ablating approximately 5.5% liver volume]), or a 35% partial hepatectomy (PHx), or a sham operation (n = 7, in each group) and followed for survival studies. In order to evaluate the potential tumorigenic effect of RFA, additional untreated (n = 12) or RFA treated (n = 12) MDR2 KO littermates were sacrificed 7 days and 1 month post the operation, and lesions greater than 3 mm in diameter were counted. In order to inhibit umorigenesis, MDR2 KO mice were treated with a c-met inhibitor or DMSO (n = 7, each) post RFA and were sacrificed 1 month post treatment.
Results: Both RFA and PHx mice survived significantly less than the sham operated mice ($p = 0.03$). Six months post treatment, only 68% of the mice that underwent RFA or PHx survived in comparison to the sham operation in which 100% of the mice were still alive. Moreover, one month post RFA, RF treated mice had and average of 1.66 dysplastic nodules compared to 0.636 in the controls ($p = 0.016$), with no significant differences noted 7 days post ablation. These lesions were observed in 83.3% of the mice that underwent RFA in comparison to 50% of the control mice ($p = 0.002$). Seven days post ablation, massive accumulation of activated myofibroblasts was observed at the border zone of the coagulation necrosis area, with increased Ki67 positive hepatocytes staining both in the ablated as well as in the untreated lobes ($p = 0.003$ and $p = 0.02$ respectively). Treatment with PHA 665752, a c-met inhibitor, post RFA diminished the tumorigenic effect of RFA in comparison to the control (tumor load: $p = 0.001$, tumor volume: $p = 0.02$).
Conclusions: RFA in an inflamed liver encounters a pro-tumorigenic effect per-se. This effect is mediated by c-met. Combining RFA and

a c-met inhibition may decrease tumor recurrence and increase survival in RFA treated HCC patients.

P0295

CD4+CD25+CD127LOW REGULATORY T CELLS PLAY PREDOMINANT ANTI-TUMOR SUPPRESSIVE ROLE IN HEPATITIS B VIRUS ASSOCIATED HEPATOCELLULAR CARCINOMA

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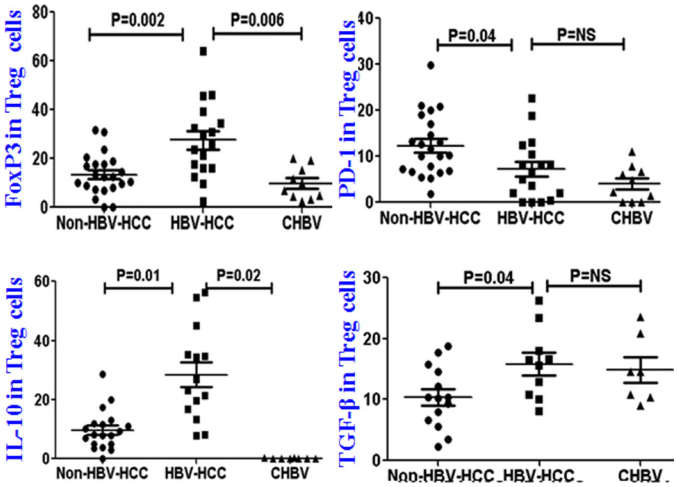
Background and Aims: Hepatocellular carcinoma (HCC) is the second leading cause of cancer death worldwide and hepatitis B is one of the common causes. T regulatory cells (Tregs) are strong immunomodulators and are likely to play major role in HCC development. We hypothesize that HBV infection expands Treg mediated immunosuppression creating ideal microenvironment for oncogenic transformations. Therefore, we investigated T regulatory cells CD4+CD25+CD127^{low} and their regulation by TGF-β, IL-10 and PD1 in patients with HBV related HCC (HBV-HCC) vs. non-HBV-HCC.
Methods: HBV-HCC (n = 17), non-HBV-HCC (n = 22; NASH =16, alcohol related=6) and Chronic hepatitis B infection (CHBV; n = 10) patients were recruited for study. Whole blood immunophenotyping was done by multicolor flow cytometry. Functionality of isolated CD4+CD25+CD127low Treg was assessed by *in vitro* suppression assay. Expression of FoxP3, IL-10, PD-1, TGF-β and Notch in Tregs and liver explants was analyzed by flow cytometry, immuno-histochemistry (IHC) and qRT-PCR.
Results: Total lymphocyte count and CD8+Tcells were significantly lower in HBV-HCC compared to Non-HBV-HCC (p = 0.04 and p = 0.04) and CHBV (p = 0.003 and p = 0.05). Foxp3 expression in CD4+CD25+^{hi} CD127^{low} was significantly increased in HBV-HCC compared to Non-HBV-HCC and CHBV patients (P = 0.002, P = 0.006). In *in vitro* suppression assay Tregs isolated from HBV-HCC showed increased suppressive ability and secretion of immunosuppressive cytokines IL-10 and TGF-β compared to Non-HBV-HCC (P = 0.01 and P = 0.04) and CHBV (P = 0.02 and P=NS). PD1 expression in CD4+CD25+^{hi} was significantly decreased in the HBV-HCC than non-HBV-HCC and CHBV (P = 0.04 and P=NS). In HBV-HCC, α fetoprotein (AFP) levels were significantly high (median 941, range 2–575736.6) than Non-HBV-HCC (median 13.5, range 2–18,900). In HBV-HCC Foxp3 expression in CD4+ CD127^{low}CD25+^{hi} was significantly correlated with both, high (>1000 ng/ml, r = 0.914, P = 0.000) and low (<1000 ng/ml, r = 0.857, P = 0.014) AFP levels. Reduced PD1 expression in HBV-HCC also had negative correlation with FOXP3 in CD4+CD25+^{hi} CD127^{low} (r = −0.78, p = 0.04).



Conclusions: Our results demonstrates that CD4+ CD25+^{hi} Tregs from HBV-HCC patients have decreased expression of PD-1, resulting in higher IL-10 and TGF-β secretion. Increased suppressive ability of Tregs in HBV related HCC confers increased anti-tumor suppressive response than in non HBV-HCC. Modulation of T-regs and PD1 may serve as useful therapeutic targets.

P0296

eNOS POLYMORPHISMS IN RELATION TO OUTCOME IN ADVANCED HCC PATIENTS RECEIVING SORAFENIB

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Background and Aims: Cancer cells adapt to hypoxic microenvironment through the activation of many molecules, including endothelial nitric oxide synthase (eNOS). Sorafenib, by blocking the vascular endothelial growth factor receptors (VEGFRs), induces an inhibition of eNOS activitywith a consequent decrease of the production of nitric oxide (NO). NO is associated with an increase of tumor angiogenesis, tumor invasion and metastasis formation. In our study we analysed the role ofeNOS polymorphisms in relation to clinical outcome in patients with hepatocellular carcinoma treated with sorafenib.
Methods: From a database of 257 patients diagnosed with hepatocellular carcinoma from 2004 to 2014, we selected 54 patients who received sorafenib. Peripheral blood samples or FFPE tumor tissues were available for DNA extraction and genotyping analysis. Three eNOS polymorphisms (eNOS +894 G/T, eNOS VNTR 27bp 4a/b, eNOS −786 C/T) were analyzed by direct sequencing or Real Time PCR method. We analyzed 21 patients for the VNTR 4a/b polymorphism and 32 patients for −786 C/T polymorphism. All the candidate genotypes were evaluated to identify a potential correlation with overall survival (OS) (log-rank test).
Results: With regard to eNOS VNTR it was observed that patients carrying the b allele (5 repetitions of 27bp) both in homozygosity (4bb) and in heterozygosity (4ab) were associated with a better OS. The variants 4aa (4 repeats of 27bp in homozygosity), 4ab and 4bb, were associated with a median OS of 5.7, 13.9 and 23.6 months, respectively (p = 0.016). For *eNOS* −786 the presence of the T allele both in homozygosity (TT) and in heterozygosity (TC) was associated with a statistically significant longer OS with respect to patients with CC genotype (15.6 versus 13.9 months, respectively, p = 0.031). No correlations were observed in relation to PFS (p = 0.494).
Conclusions: eNOS VNTR and eNOS −786 could represent prognostic markers in patients with advanced hepatocellular carcinoma treated with sorafenib.

P0297

THE TRANSFORMING GROWTH FACTOR-BETA (TGF-β) GOVERNS HUMAN LIVER TUMOR CELL PLASTICITY

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Background and Aims: Transforming Growth Factor-beta (TGF-β) acts as a tumor suppressor in initial stages of hepatocarcinogenesis. However, tumour cells acquire mechanisms to overcome TGF-β suppressor effects and respond to it undergoing epithelial–

mesenchymal transition (EMT). In some tumour cells a link exists between EMT and the expression of stem cell markers. Aim of this study was to analyze if autocrine activation of the TGF-β pathway in hepatocellular carcinoma (HCC) cells may control the expression of Cancer Stem Cell (CSC) markers concomitant with the acquisition of mesenchymal properties.

Methods: Different HCC cell lines with different autocrine expression of TGF-β and epithelial–mesenchymal phenotypes were analysed. TGF-β Receptor I (TβRI) expression was stably silenced by shRNA. EMT and SC marker expression was analyzed by Flow Cytometry, Immunofluorescence and Real-Time PCR. Sphere-forming and colony-formation assays were performed to explore the biological properties of liver CSCs.

Results: Epithelial liver tumour cells expressed EpCAM and CD133, whereas mesenchymal-like cells expressed CD44. Cancellation of the TβRI in Hep3B (with a mixed epithelial–mesenchymal phenotype) prevented the TGF-β-induced EMT, but also induced a mesenchymal–epithelial transition (MET). Relevantly, TβRI knock-down decreased the basal expression of EpCAM and CD133 and reduced the formation of liver spheroids and number of clones. The TβRI knock-down in HLE and HLF (mesenchymal-like cells) decreased significantly the expression of the Snail family genes, but unexpectedly did not produce a full MET. Nevertheless, TβRI knock-down decreased expression of CD44, which correlated with a lower ability to form liver spheroids and clones. Interestingly, chronic treatment of Hep3B cells with TGF-β induced progressive down-regulation of EpCAM and CD133 and up-regulation of CD44, concomitant with the appearance of a mesenchymal phenotype. Furthermore, these cells formed liver spheres and colonies more efficiently.

Conclusions: TGF-β not only modulates the EMT phenotype of HCC cells, but also the expression of CSC genes, although it is not clear yet the cross-talk among both processes. A mesenchymal phenotype and CD44 expression are associated with poor prognosis in HCC. Results of this study further support that activation of the TGF-β pathway may be considered a therapeutic target in HCC.

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P0298

GLUCOSE TRANSPORTER ISOFORM 1 (GLUT1) EXPRESSION DETERMINES HEPATIC METASTASIS OF MELANOMA CELLS

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Background and Aims: The facilitative glucose transporter isoform 1 (GLUT1) is the key rate-limiting factor in glucose transport into cancer cells, and we have previously shown that GLUT1 is a tumor-promotor in hepatocellular carcinoma, while its expression is at the detection limit in normal hepatocytes.

The aim of this study was to analyze whether GLUT1 expression and a high capacity for glucose uptake, respectively, are general pro-cancerogenic factors in the liver.

Methods: We used malignant melanoma as a model-tumor, which is known to preferentially metastasize to the liver.

Results: Similar as observed in HCC, GLUT1 expression was enhanced in melanoma cell lines compared to primary melanocytes, as well as in melanoma compared to naevi. Immunohistochemical analysis of a tissue micro array consisting of 140 human melanoma tissues showed that GLUT1 expression was significantly enhanced in metastasis compared to primary tumors. GLUT1 expression in primary tumors correlated with tumor staging, and most importantly, with progression- and overall-survival. To determine the role of GLUT1 in melanoma metastasis, GLUT1 expression was suppressed in the murine melanoma cell line B16 (i) by stable transfection with shRNA and (ii) by using the selective GLUT1-Inhibitor WZB117. GLUT1 suppression caused decreased anaerobic glycolysis and lactate secretion, and inhibited proliferation and migration of B16 cells. Moreover, GLUT1 suppression lowered apoptosis resistance of melanoma cells. Next, B16 cell clones with and without GLUT1 suppression were subjected to an established model of hepatic metastasis, in which tumor cells were injected into the spleen of syngeneic mice, from where they metastasize into the liver *via* the portal circulation. GLUT1 suppressed cells formed significantly less metastases and hepatic metastases derived from GLUT1 suppressed B16 cells revealed less immune-cell infiltration and more apoptosis as assessed by CD3-immunohistochemistry and TUNEL staining.

Conclusions: Our data promote the hypothesis that high glucose levels in the portal circulation and the liver, and the capacity to utilize those, respectively, promote hepatic metastasis. Our data indicate enhanced apoptosis resistance of tumor cells and known immunomodulatory effects of lactate as potential underlying mechanisms of this phenomenon. GLUT1, which is almost selectively expressed in malignant cells but not in healthy liver or other non-malignant tissues, appears as an attractive therapeutic target for hepatic metastasis.

P0299

SORAFENIB EFFECT ON MITOCHONDRIAL FUNCTION PROVIDES A TARGET FOR INCREASING HCC THERAPY EFFICACY

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Background and Aims: Multikinase inhibitor sorafenib has limited efficacy in the treatment of advanced hepatocellular carcinoma (HCC). Novel therapies, in combination with sorafenib or in monotherapy, are demanded to increase drug efficacy in HCC treatment. The lack of positive results from other drugs, underscores the importance of identifying weaknesses in HCC biology that current approaches have not recognized. A mitochondrial effect of sorafenib has been previously reported, although its participation in sorafenib toxicity and HCC therapy has drawn little attention.

Methods: Hepatoma cell lines (HepG2 and Hep3B) were treated with sorafenib and Bcl2-inhibitors. Western blots in total, cytosolic and mitochondrial extracts and qPCRs were performed after sorafenib exposure. ROS production, mitochondrial membrane permeabilization, caspase activity and ATP measurements were analyzed in sorafenib-treated hepatoma cells. Tumor growth was determined after subcutaneous injection of HepG2 cells on the flanks of nude mice.

Results: Sorafenib induces a rapid decline in mitochondrial membrane potential, with production of reactive oxygen species (ROS) and reduction in the levels of the antiapoptotic Bcl-2-family protein MCL-1. However, cytochrome c release from mitochondria and/or ATP depletion after sorafenib exposure were not observed during hours, suggesting modest mitochondrial contribution in cell death. Bcl-2 levels, that play an important role in mitochondrial dependent cell death, were not affected by sorafenib, so we decided to evaluate the effect of several Bcl-2 inhibitors (HA-14, ABT-263, ABT-767 and AT-101) on sorafenib toxicity in hepatoma cells. Among them, ABT-263 (Navitoclax) a potent inhibitor of Bcl-xL and Bcl-2 now in Phase II clinical trials for leukemia and solid tumors, was the drug that exhibited higher capacity to potentiate sorafenib effects on Hep3B and HepG2 cells. ABT-263 co-administration with sorafenib induced quick release of cytochrome c and enhanced caspase-3 activity. Moreover, *in vivo* administration of ABT-263 combined with sorafenib greatly potentiated sorafenib effects,

decreasing subcutaneous tumor growth of HepG2 cells in nude mice.
Conclusions: Sorafenib alone is unable of inducing mitochondrial dependent death but provokes mitochondrial vulnerability that allows Bcl-2 inhibitor ABT-263 to sensitize hepatoma cells to sorafenib treatment. Navitoclax increases sorafenib efficacy and Bcl-2 inhibitors should be taken into account in HCC management.

P0300
DICHOTOMY OF TRANSFORMING GROWTH FACTOR-β SIGNALING IN HEPATOCELLULAR CARCINOMA PROGRESSION

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Background and Aims: Transforming growth factor (TGF)-β is a ubiquitously expressed cytokine with fundamental roles in various aspects of cell physiology. In carcinogenesis, TGF-β signaling plays a dual role. While it suppresses the proliferation of epithelial cells and adenoma cells at early stages by inducing growth arrest and apoptosis, it triggers epithelial to mesenchymal transition (EMT) and gain of metastatic abilities at later stages of carcinoma development. The molecular mechanisms underlying this 'TGF-β-switch' are only beginning to be unravelled. To mimic the pathophysiological situation as closely as possible, we exposed the cells to TGFβ long-term. Through this approach we aimed to identify those cooperating factors and signaling pathways that cause HCC cells to interpret the TGF-β signal in a tumor progressive way.
Methods: *In vitro* comparison of migratory behaviour of various HCC cell lines treated long-term (>10 days) with TGF-β. Analysis of regulatory networks and target genes underlying the TGF-β treatment.
Results: HCC cell lines that have undergone EMT secrete TGF-β and show elevated levels of Smad2/3 phosphorylation indicating an autocrine regulatory feedback loop. Inhibition of TGF-β by LY2109 abrogates autocrine stimulation and diminishes the migratory potential of mesenchymal HCC cells. Silencing of either TGF-βR1 or Smad4 indicated the importance of canonical TGF-β/Smad signaling HCC cell migration. Short-term treatment of cells with TGF-β could not improve migratory abilities. Interestingly, long-term TGF-β treatment revealed crucial differences between mesenchymal HCC cell lines. While HLF cells showed an increase in migration when treated with TGF-β for more than 10 days, SNU449 displayed a dramatic reduction in migration. However, both cell lines displayed no modulation in Smad phosphorylation, indicating a change in the utilization of TGF-β signaling in long-term treated SNU449 cells.
Conclusions: EMT-transformed HCC cells establish an autocrine TGF-β loop which stimulates migration. However, TGF-β cannot add up to the autocrine loop but causes a different, even opposing reaction, over time. Interpretation of long-term TGF-β signaling, which mimics the patient's situation more closely, depends on duration and intensity and is controlled by co-acting factors and signaling pathways.

P0301
TARGETING OF MIR-518D REDUCES CHEMORESISTANCE IN HEPATOCELLULAR CARCINOMA CELL LINES

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Background and Aims: Glycine N-methyltransferase (GNMT) catalyzes the conversion of S-adenosylmethionine (SAM) to glycine generating S-adenosylhomocysteine and sarcosine. This enzyme is highly abundant in the adult liver, and its expression has been observed downregulated or completely blocked in liver and prostate tumors, in most cell lines and in some preneoplasic lesions. The transient expression of the protein in cancer cell lines induces apoptosis.
MicroRNAs (miRNAs) are small noncoding RNAs that selectively target mRNAs leading to a translational repression or mRNA degradation. The dysregulation of specific miRNAs leads to chemoresistance in different cancers and correction of these miRNAs can sensitize cancerous cells to chemotherapy.
In this study we found a miRNA (miR-518d) that reduces GNMT level providing chemoresistance to a variety of cancer cell lines.
Methods: The candidate miRNAs regulating GNMT expression were found by in silico analysis (Targetscan and EMBL-microcosm). After this, the expression of candidate miRNAs was tested by real-time PCR in metastatic and hepatocellular carcinoma human samples, and in tumoral cell lines with different chemoresistance level. Finally, the resistance to doxorubicin and sorafenib treatments was assessed in tumoral cell lines after overexpressing or silencing the miRNA.
Results: Two miRNAs (miR-873 and miR-518d) were found to regulate GNMT expression. The analysis of both miRNAs in metastatic and hepatocellular carcinoma human samples found a miR-518d elevated in comparison with healthy samples.
miR-518d and GNMT levels were measured in sensitive and chemoresistant cell lines. It showed a correlation in which the drug-resistant cell lines presented higher amount of miR-518d and lower GNMT.
Finally, the overexpression of miR-518d in HuH7 hepatoma cell line increased the resistance to doxorubicin, whereas the inhibition of the miRNA in BCLC3 hepatoma cell line sensitized it to doxorubicin and sorafenib.
Conclusions: miRNA-518d is increased in tumoral samples and hepatoma cell lines, reducing GNMT expression. The expression of this miRNA correlates with the chemoresistance, and its inhibition sensitizes the hepatoma cell lines to treatment with drugs such as doxorubicin and sorafenib. The selective modulation of this microRNA can be important to improve the response to chemotherapies, sensitizing the tumors to the specific drugs.

P0302
CONSTITUTIVE GP130 ACTIVATION ACCELERATES TRANSFORMATION OF PROLIFERATING HUMAN HEPATOCYTES VIA INCREASED LEVELS OF OXIDATIVE STRESS

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Background and Aims: Presently, a prominent hypothesis states that pro-inflammatory signaling pathways (IL-6) and reactive

oxygen species (ROS) promote hepatocyte transformation during the course of chronic hepatitis. In order to elucidate the driving oncogenic mechanisms, we activated the IL-6 signal transducer glycoprotein 130 (gp130) in untransformed hTERT-immortalized human fetal hepatocytes (FH-hTERT) and challenged the cells with ROS.

Methods: We generated FH-hTERT clones with stable expression of a ligand-independent constitutively active gp130 construct (L-gp130). Following phenotype characterization, we treated FH-hTERT/L-gp130 clones with H_2O_2 after glutathione depletion with BSO. ROS levels were measured (Carboxy-H_2DCFDA) and DNA-double strand breaks (DSB) were determined by immunofluorescent staining for γ-H2AX and Rad51. We assessed the expression of DNA-damage and antioxidant response genes by qRT-PCR. Proliferation was monitored by BrdU incorporation, and anchorage-independent growth was scored in soft agar. Finally, to compare ligand-independent with ligand-dependent IL-6 signaling, we activated IL-6 transsignaling with the designer cytokine Hyper-IL-6 and recapitulated the experiments.

Results: Constitutive gp130 activation alone was not sufficient to induce full malignant transformation. However, challenge with H_2O_2/BSO resulted in 2- to 3-fold higher ROS levels and up to 5-fold more DSB in FH-hTERT/L-gp130 clones in comparison to parental cells. Despite higher ROS levels, FH-hTERT/L-gp130 clones survived and even displayed an enhanced proliferation following treatment with H_2O_2/BSO, and developed colony growth capabilities in soft agar with a frequency of up to 20 colonies per 5,000 seeded (FH-hTERT with/without treatment no colonies, FH-hTERT/L-gp130 clones without treatment no colonies). As possible mechanism, we detected a decreased expression of antioxidant genes, like GPX3 and APOE, and absence of p21 upregulation. In contrast to FH-hTERT/L-gp130 clones, FH-hTERT incubated with Hyper-IL-6 did not demonstrate increased ROS levels and, as observed in control cells, proliferation was diminished in response to oxidative stress.

Conclusions: In contrast to Hyper-IL-6 stimulation, ligand-independent constitutive activation of gp130 resulted in an enhanced cell cycle turnover despite increased levels of ROS and DSB. Increased ROS levels in hepatocytes might be a consequence of an altered gp130-mediated oxidative stress response.

P0303

HEPATITIS C VIRUS CORE PROTEIN INCREASES OCT4 EXPRESSION AND PROMOTES CELL CYCLE PROGRESSION IN HEPATOCELLULAR CARCINOMA

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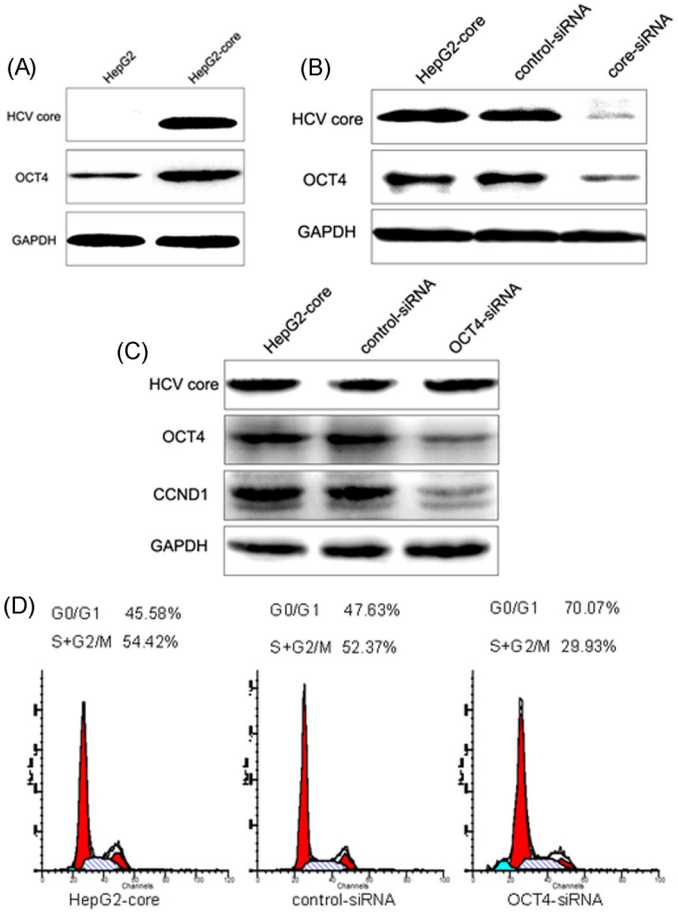
Background and Aims: Hepatitis C virus (HCV) core protein plays an important role in the development of hepatocellular carcinoma. OCT4, a homeodomain transcription factor of the POU family, is critically involved in the self-renewal of undifferentiated embryonic stem cells. Abnormal expression of OCT4 has been detected in several human solid tumors. However, relationship between HCV core and OCT4 remains uncertain. The aim of this study was to investigate the role of HCV core in regulating OCT4 expression and its role in tumorigenesis of hepatocellular carcinoma.

Methods: HepG2 stable cell lines were established by transfection with pcDNA-core. HCV core siRNAs were employed to knockdown of HCV core expression. OCT4 expression was analyzed by qRT-PCR and Western blot. Proliferation and cell cycle of HepG2 cells, transfected with OCT4 siRNAs, were assessed using CCK8 assay and flow cytometry.

Results: The results showed that HCV core efficiently up-regulated OCT4 expression (Fig. A). The expression levels of OCT4 at both mRNA and protein were markedly increased in HepG2 cells with HCV core transfection. Meanwhile, HCV core-increased OCT4 expression was attenuated by RNA interference-mediated knockdown of HCV core (Fig. B). In addition, HCV core-increased OCT4 expression resulted in enhanced cell proliferation and cell cycle progression. Inhibition of OCT4 reduced the CCND1 expression and induced G0/G1 cell cycle arrest in hepatocellular carcinoma cells (Fig. C,D).

Conclusions: Our findings suggest that HCV core protein can increase OCT4 expression and promote cell cycle progression, and also provides a new insight into the mechanism of hepatocarcinogenesis by HCV infection.



P0304

THREE DIMENSIONAL CULTURE OF HepG2 LIVER CELLS ON A RAT DECELLULARIZED LIVER MATRIX AS A MODEL FOR HEPATOCELLULAR CARCINOMA PATIENTS

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Background and Aims: Three-dimensional *in vitro* tumor models are needed to obtain more information about drug behavior in tumors. The aim of this study is to establish a new model for hepatocellular carcinoma (HCC) using decellularized rat livers.

Methods: After generating acellular rat liver matrix, characterization was performed in terms of morphology, ultrastructure and biochemistry. HepG2 liver cancer cells were perfused via the portal vein and placed in a bioreactor for 10 days. Histology was performed to analyze cell distribution within the scaffolds. Function and tumor-related gene expression were examined by polymerase chain

reaction (PCR), immunohistochemistry and western blot. Albumin secretion by cells grown within scaffolds, monolayer culture and on Matrigel was chekced by ELISA. We also evaluated the function of HepG2 cells grown on scaffolds in the presence of a well-known anti-cancer drug; methotrexate, to investigate the potential application of our system for drug screening.

Results: Histological and scanning electron microscopy examinations of decellularized scaffold revealed total removal of the cytoplasmic and nuclear materials. Perfusion of decellularized liver by fluroscent dextran showed the preservation of the vascular tree in scaffolds. Biochemical analysis showed the presence of a negligible amount of DNA and preserving of the important extracellular matrix components such as collagen, elastin and glycosaminoglycans. HepG2 cells grew well on the scaffolds. PCR, immunohistochemical examination and western blotting showed the ability of HepG2 cell grown within scaffolds to maintain their function and tumorgenicity at significantly higher levels than cells grown on two-dimensional (2-D) dishes or spheroids on Matrigel. Unlike the 2-D cultures, albumin secretion and alpha-fetoprotein expression in three-dimensional cultures were less susceptible to lower concentrations of the drug. Cells grown in scaffolds seemed to respond to the drug in an analogous manner to its known activity *in vivo*.

Conclusions: In summary, using HepG2-reseeded scaffolds as *in vitro* 3-D cancer models provided a superior 3-D cell culture environment for tumor studies and will enable researchers to evaluate and predict the efficacy of new anti-cancer drugs.

P0305
HOXA13 EXPRESSION IS ASSOCIATED TO WORST PROGNOSIS IN HCC AND MODULATES HCC-DERIVED CELLS RESPONSE TO SORAFENIB IN VITRO

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Background and Aims: Despite significant advances in HCC diagnosis and management, for advanced stages no therapeutic options exist beside Sorafenib. Recently, using human liver biopsies, we showed that HOXA13 expression in HCC correlates with poor survival and metastasis presence. In addition, we observed that HOXA13 expression increases HCC cells proliferation *in vitro*. Here we seek to confirm our data on a larger cohort of samples and to investigate whether HOXA13 could modulate cells' Sorafenib response.

Methods: A liver TMA (tissue microarray) comprises a total of n = 305 specimens, n = 82 normal liver tissues, n = 108 cirrhotic patients and n = 115 HCCs, has been stained for HOXA13, CK-7, CK-19, E-Cad. Protein levels have been correlated with patients'clinical data. *In vitro* experiments to stably modulate HOXA13 expression (gain and loss of function) have been performed using the HCC derived cell lines: Hep-G2, SNU449 and PLC5. Subsequently, cells have been treated with Sorafenib and cell cycle analysis, proliferation, migration and drug responce have been tested.

Results: HOXA13 is altered in 41% of HCC tested samples, thus confirming our previous results obtained using iver biopsies. In addition, high HOXA13 levels are associated with poorer outcome and higher grading (Edmondson and BCLC). Increased HOXA13 expression is linked with stem progenitor markers, CK-7 and CK19. Furthermore, high HOXA13 expression is coupled with diminished levels of E-Cad, providing a molecular basis for its association with metastasis in HCC. Finally, *in vitro* experiments demonstrate that HOXA13 overexpression results in higher resistance to Sorafenib exposure. Conversely, HOXA13 downregulation sensitize HCC cells to Sorafenib.

Conclusions: Here we show that HOXA13 IHC-based protein levels can reliably predict HCC outcome and correlates with a number of tumour features (e.g. grade). Thus the usage of HOXA13 as a marker for HCC aggressiveness deserves further investigations. In addition, our *in vitro* data concerning HOXA13 modulation of Sorafenib response prompt us to validate this finding *in vivo* using HCC samples.

P0306
IMPAIRMENT OF AUTOPHAGY IN THE EARLY STAGES OF HEPATOCARCINOGENESIS

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Background and Aims: Hepatocellular carcinoma (HCC), the third leading cause of cancer-related mortality worldwide, represents a major health problem. Recent studies highlight the importance of autophagy, a catabolic process by which cells remove protein aggregates and damaged organelles, in hepatocarcinogenesis. Autophagy dysfunction is also associated with neurodegeneration, aging and autoimmune disorders, however, its role in carcinogenesis remains complex, dependent on cancer stage and still controversial. Therefore, the aim of this study was to investigate the role of autophagy in the early stages of HCC development.

Methods: The Resistant-Hepatocyte (R-H) rat model [1], which offers the possibility to identify distinct lesions (preneoplastic nodules, early and fully advanced HCCs) at well-defined timings, was used. Autophagy was investigated by immunohistochemistry, electron microscopy and qRT-PCR.

Results: First of all, the expression of different autophagy markers (Ambra1, p62, Becn1, VpS34, UVRAG) was investigated in preneoplastic nodules. The results indicate that while the mRNA levels of Ambra1, p62, Becn1, Ulk1, Atg5 and Atg12 were not increased in the preneoplastic lesions, immunohistochemistry analysis demonstrated a marked accumulation of all analyzed proteins in preneoplastic nodules when compared with the surrounding tissue, suggesting impairment of the autophagic process. The results of electron microscopy analysis confirmed the autophagy blockage, as demonstrated not only by the lack of autophagic vacuoles but also by the presence of strongly altered, swollen and unremoved mitochondria. Recent studies [2] reported that p62 interacts with the Nrf2-binding site on Keap1, resulting in stabilization of Nrf2 followed by transcriptional activation of its target genes. Accordingly, p62 accumulation in preneoplastic nodules was associated with a significant induction of NQO1, GCLC and GSTA4 (Nrf2 target genes). Finally, the expression levels of miRNA-224, a miR whose expression is inversely correlated with autophagy [3], was about 100-fold higher in preneoplastic nodules when compared to the surrounding tissue.

Conclusions: These results strongly suggest that autophagy results impaired in the early stages of hepatocarcinogenesis induced by R-H model and its modulation may represent a promising therapeutic target in HCC.

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P0307

MELATONIN-INDUCED APOPTOSIS OF HepG2 CELLS IS ENHANCED BY AUTOPHAGY SUPPRESSION

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Background and Aims: Hepatocellular carcinoma is the five most common malignancy worldwide. Autophagy is a process that targets internal or damaged organelles and misfolded proteins to lysosomal degradation for the purpose of maintaining homeostasis during stress, although this process also contributes to cell death in several situations. On the other hand, ceramides have emerged as important effectors in the regulation of the autophagic pathway, mediating the cross talk between apoptosis and autophagy. Melatonin induces anti-apoptotic, anti-angiogenic and anti-invasiveness effects on HepG2 cells. However, it is unknown how autophagy affects cell death induced by melatonin. The aim of this study was to explore the effects of melatonin on autophagy and cell death mechanisms in HepG2 cells.
Methods: Autophagy was evaluated in HepG2 cells by Western blot of LC3 and p62 and confocal microscopy of LC3 and LAMP2. Autophagy flux was assessed using 50 μM chloroquine and 100 nM bafilomicyn. Ceramide levels were measured by HPLC, and acid sphingomyelinase (ASMase) activity using a fluorescent sphingomyelin analog. mRNA levels were assessed by RT-PCR and cell viability by MTT.
Results: Melatonin administration (1–2 mM) induced a transient autophagic response characterized by LC3II increased expression and p62 degradation, as well as LC3II and LAMP2 colocalization, which reached a maximum at 8 hours and disappeared at 24 h. Poly-ADP-ribose-polymerase (PARP) cleavage and BAX protein levels increased while cell viability was reduced from 24 h to 48 h of treatment. ATG5-silenced HepG2 cells were more susceptible to melatonin induced-cell death than non-silenced cells, and showed a significantly increased cleavage of PARP. Melatonin was able to enhance ceramide levels, serine palmitoyltransferase (SPT) and acid sphingomyelinase (ASMase) expression, and ASMase activity after 4 h of administration. SPT inhibition (5 μM myriocin) prevented melatonin induced autophagy and there was an impaired autophagy flux in response to the ASMase inhibitor imipramine (20 μM).
Conclusions: Melatonin induces in HepG2 cells an early and transient autophagic response that is prevented by ceramide inhibitors. Furthermore, suppression of autophagy by ATG5 silencing makes cells more susceptible to melatonin-induced apoptosis. Our findings support a protective effect of autophagy, partially regulated by ceramides, on melatonin-induced apoptosis, and suggest that autophagy suppression may strengthen the proapoptotic effect of melatonin in liver cancer

P0308

GENOME-WIDE HIGH-THROUGHPUT SCREENING OF TGF-BETA RELATED BIOMARKERS IN HCC CELLS

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Background and Aims: The small molecule kinase inhibitor, LY2157299, targeting the TGF-β receptor I (RI), blocks invasion and migration of HCC cells in vitro and is currently being evaluated in clinical trials, where it shows promising anti-tumor effects in HCC patients. Aim of the study is to identify new biomarkers to assess the drug's efficiency.
Methods: The mRNA and smallRNA transcriptomes of an HCC cell line treated with TGF-beta and LY2157299, used both alone and in combination, were investigated using Next-Generation Sequencing (NGS)-based Massive Analysis of cDNA Ends (MACE) and smallRNA seq, respectively. For functional assignment of differentially expressed genes, Gene Ontology enrichment analysis was performed. Differential expression of selected TGF-β-responsive transcripts and microRNAs that were reverted by LY2157299 to the level of the untreated controls were evaluated by Quantitative Real-time (qRT-) PCR. The same assays were used to analyse the expression of the respective mRNAs and miRNAs in HCC patients.
Results: Six mRNA transcripts were selected as significantly up-regulated by TGF-β and down-regulated by LY2157299. Some of these transcripts, such as *PMEPA1*, are highly expressed also in other cancers, being targets for therapeutic drugs. Others, such as *SKIL* and *Snai1,* are involved in the TGF-β/SMAD pathway and evoke tumor progression in HCC. We also detected, for the first time, that the *C4orf26* transcript was significantly up-regulated by TGF-β in our cell line. We further identified three novel microRNAs, namely miR-27a-5p, miR-1248 and miR-1973, that were up-regulated by TGF-β and responded to LY2157299 treatment. miR-27a regulates lipid metabolism and cell proliferation during hepatic stellate cell (HSC) activation, affecting the expression of 1267 proteins. However, nothing is known about the relation of these miRNAs to TGF-β signaling. In HCC patients, *ANGPTL4*, *SNAI1*, *SKIL* and *PMEPA1* mRNAs are strongly expressed in tissue specimens, and the last three are also significantly correlated with TGF-b1 mRNA levels.
Conclusions: We identified at least one novel mRNA and 3 novel miRNAs that have not previously been related to TGF-β signaling and may be important for TGF-β-related oncogenicity. Further in vivo studies are needed to investigate their role in HCC progression and also as biomarkers for companion diagnostics of LY2157299 treatment in HCC patients.

P0309

GENE EXPRESSION PROFILING OF HEPATOCELLULAR CARCINOMA BIOPSIES REVEALS THREE MOLECULAR CLASSES WITH DISTINCT CLINICAL AND BIOLOGICAL PROPERTIES

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Background and Aims: Hepatocellular carcinoma (HCC) is a heterogeneous disease, and despite considerable research efforts, no molecular classification of HCC has been introduced in clinical practice. The existing molecular classification systems were established using resected tumors, which introduces a selection bias towards patients without liver cirrhosis and with early stage HCCs. So far, these classification systems have not been validated in liver biopsy specimens from tumors diagnosed at intermediate and late stages.
Methods: We generated and analyzed expression profiles of 60 HCC biopsies from an unselected patient population representing all tumor stages.
Results: Unbiased clustering identified 3 HCC classes. Class membership correlated with survival, tumor size, and with Edmondson and BCLC stage. Most biopsy specimens could be assigned to the classes of published classification systems, demonstrating that gene expression profiles obtained from patients with early stage disease are preserved in all stages of HCC. When a reference set of healthy liver samples was integrated in the analysis, we observed that the differentially regulated genes

up- or down-regulated in a given class relative to other classes were actually dysregulated in the same direction in all HCCs, with quantitative rather than qualitative differences between the molecular subclasses. With the exception of a subset of samples with a definitive β-catenin gene signature, biological pathway analysis could not identify class specific pathways reflecting the activation of distinct oncogenic programs.
Conclusions: Our results suggest that gene expression profiling of HCC biopsies has limited potential to direct therapies that target specific driver pathways, but can identify subgroups of patients with different prognosis.

P0310
FAK/EZH2 FUNCTIONAL INTERACTION IS CRUCIAL FOR HCC DEVELOPMENT AND PROGRESSION BOTH IN VIVO AND IN VITRO

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Background and Aims: Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the incidence is rising across most of the world. During the multi-step process of HCC pathogenesis, the activation/inactivation of several epigenetic mechanisms, mainly those regulating proliferation/apoptosis and migration, may play a major role. Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase involved in the regulation of many cellular processes such as proliferation, adhesion, invasion or cell survival. FAK over-expression and activation, often occurs during neoplastic transformation including HCC development, even though its role during hepatocarcinogenesis remains unclear. The aim of the present study was to investigate the effect of FAK silencing in an *in vivo* HCC xenograft NOD/SCID mouse model and in an *in vitro* model of HCC.
Methods: We established HepG2Luc-siCTRL or HepG2Luc-siFAK cells that were intrahepatically injected in NOD/SCID mice to analyze *in vivo* tumorigenicity or used to study the role of FAK on HepG2 cell homeostasis. BrdU incorporation, cell proliferation and soft agar assays were performed to assess the effect of FAK silencing on cell tumorigenic ability. Real-Time PCR, Western Blotting and immunofluorescence assays were also carried out to study the expression and the activity of FAK and EZH2 in FAK depleted cells.
Results: Xenograft experiments showed a range of 30–60% reduction on the tumor mass derived from HepG2Luc-siFAK injection with respect to that observed in HepG2Luc-siCTRL cells. In HCC from silenced HepG2 clones the expression of FAK and PCNA was drastically down-regulated. Furthermore, FAK-silenced HepG2 cells showed a statistically significant downsized *in vitro* proliferation and invasion rates. The silencing of FAK also induced a reduction of major pathways regulating hepatocellular homeostasis. Interestingly, a down-regulation of EZH2 at the transcript level was observed in FAK-silenced cells together with a decreased nuclear EZH2 and a reduced expression of the 3meH3k27. We also reported an increased expression of the EZH2-targeted mir-200b compared to control cells.
Conclusions: In conclusion our results showed that FAK silencing can counteract the invasive and high proliferative cancer phenotype in HCC. Moreover, the silencing critically affect the activity of EZH2 suggesting a potential interaction between FAK and EZH2. However, further studies are needed to shed light on FAK and EZH2 molecular cross-talk.

P0311
BALB/C AND C57/BL6 MICE EXHIBIT DIFFERENCES IN THEIR SUSCEPTIBILITY AND ANTI-TUMOR RESPONSE TO B16F10 MELANOMA LIVER METASTASIS

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Background and Aims: While progress has been made in increasing survival rates in neoplasms detected at early stages, the prognosis of metastasized tumors remains dismal. Liver metastases in particular are survival-limiting in a broad range of malignancies. Therefore, a better understanding of the mechanisms that underlie (liver) metastasis is essential for improving cancer patients' outcome. The B16F10 melanoma cell line is a well described cancer cell line with high metastatic potential that has been successfully used for human anti-cancer drug development. Since the B16F10 cell line has very low expression of MHC class I molecules, it is predestined for studying the innate anti-tumor immune response.
Methods: We administered B16F10 cells stably transfected with a luciferase-expressing lentivirus via intrasplenic injection to induce liver metastasis in two different strains of wild-type mice. Bioluminescence was recorded 7 and 14 days after injection using an IVIS in vivo imaging system. 2 weeks after injection, mice were sacrificed and their metastatic burden measured. Liver probes were analysed with regard to immune cell populations and markers of M1-/M2-polarization by FACS, RT-qPCR and IHC. Whole transcriptome sequencing was performed on selected samples and their differential gene expression analysed.
Results: Within 2 weeks, mice developed severe metastatic disease. Notably, the allogeneic mouse strain (Balb/c) was more susceptible to B16F10 liver metastasis than the syngeneic strain (C57/Bl6). Metastasized livers showed a pattern of predominantly innate immunity with macrophages representing the major immune cell population. Interestingly, Balb/c mice exhibited a pattern of M1-, while C57/Bl6 mice showed a pattern of M2-macrophage polarization. Complete RNA sequencing of metastasized liver samples demonstrated that interferon-γ and its downstream pathway (which is usually associated with an anti-tumor response) was significantly overexpressed in Balb/c mice, whereas the Tgf-beta pathway (which is considered immunosuppressive) was significantly upregulated in C57/Bl6 mice.
Conclusions: The B16F10 melanoma liver metastasis model allows to study the innate immune response to invading cancer cells in the liver. The two wild-type mouse strains, Balb/c and C57/Bl6, appear to differ dramatically in their immunological anti-tumor responses, which is currently further explored to better define therapeutic targets related to innate immunity.

P0312

PRECLINICAL EVALUATION OF DEXTRAN-BASED THERAPEUTIC NANOPARTICLES FOR HEPATIC DRUG DELIVERY

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Background and Aims: Nanoparticles bear great potential for a broad range of clinical applications. In particular, nanoparticles may be used for targeted therapies in which the active substance is specifically delivered to the desired organ, tissue or cell population increasing its therapeutic efficacy while reducing its side effects. A few examples of such therapeutic nanoparticles are already routinely used in the clinic today, e.g. liposomal amphotericin B (Ambisome®) and pegylated liposomal doxorubicin (Caelyx®). We aimed to explore novel therapeutic nanoparticles targeting the (innate) immune system for the treatment of inflammatory/fibrotic liver disease or primary/metastatic liver cancer using dextran-based nanoparticles designed for liver specific drug delivery.
Methods: Dextran nanoparticles measuring 100 to 150 nm were synthesized using a mini-emulsion technique from dextran, a polysaccharide of glucose naturally found in bacteria. Particles were loaded with (near infrared) fluorescent dyes, siRNA or small molecules. In vitro, uptake of nanoparticles in different cell types was documented by laser scanning microscopy and measured by FACS. Cell viability was quantified using the CellTiter-Glo® assay. In vivo, nanoparticles were administered to wild-type mice via retroorbital injection and their biodistribution was recorded using an IVIS in vivo imaging system. Following repeated treatments serum parameters were analysed to detect relevant toxicities. Samples from treated mice were studied by FACS, immunofluorescence and RT-qPCR
Results: In vitro, nanoparticles were readily taken up by endothelial cells, hepatocytes and macrophages with no clear preference for a specific cell type. Viability of cells was not decreased after incubation with the particles. Nanoparticles loaded with paclitaxel showed similar cytotoxicity as conventional paclitaxel. In vivo and ex vivo, nanoparticles were largely distributed to the liver (70–80%) and the lungs (10–20%) with irrelevant distribution to other organs (including the spleen). Following repeated particle injections, serum parameters indicated a lack of overt in vivo toxicity. After a single treatment, a large portion of liver macrophages (70%) had taken up the nanoparticles.
Conclusions: Dextran-based nanoparticles of defined size are multifunctional liver specific drug carriers, which lack toxic side effects and bear great potential for clinical applications targeting liver macrophages.

P0313

CORRELATION BETWEEN VEGF AND VEGF-R POLYMORPHISMS, TOXICITY AND CLINICAL OUTCOME IN HCC PATIENTS RECEIVING SORAFENIB

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Background and Aims: The introduction of sorafenib for the treatment of advanced HCC radically changed patients' clinical outcome. However response to treatment as well as toxicity are still largely unpredictable in the single patient. We previously reported that VEGF and VEGFR polymorphisms may have a predictive and prognostic role in this setting, but little is known about the possible correlation with toxicity. The aim of our study was to evaluate whether VEGF and VEGFR genotyping was able to correlate with toxicity in HCC patients receiving sorafenib.
Methods: 73 histological samples of HCC patients receiving sorafenib were tested for VEGF-A, VEGF-C and VEGFR-1, 2, 3 single nucleotide polymorphisms (SNPs). Patients time to progression (TTP), overall survival (OS) and toxicities were analysed.
Results: VEGF-A rs833061 T>C, rs699947 C>A and rs2010963 C>G polymorphisms were statistically significant associated with any grade global (respectively: $p = 0.031$; $p = 0.018$; $p = 0.003$) and cutaneous toxicities (respectively: $p = 0.043$; $p = 0.019$; $p = 0.025$). Furthermore patients with any grade global and cutaneous toxicities showed a better progression free survival and overall survival (global toxicity PFS: 7.0 vs 5.0 months, $p = 0.016$; OS: 26.8 vs 13.0 months, $p = 0.023$) (cutaneous toxicity PFS: 7.6 vs 5.1 months, $p = 0.033$; OS: 22.7 vs 13.3 months, $p = 0.014$).
Conclusions: In our analysis patients with polymorphism T at rs833061, C at rs699947 and C at rs2010963 showed a higher rate of toxicities and, accordingly to our previous report, this correlates with a better PFS and OS. Analysis of VEGF and its receptor genes polymorphisms represents a clinical tool to identify patients with favourable response to sorafenib presumably related to a more efficient control of tumour growth. The occurrence of toxicity could be an interesting clinical surrogate during sorafenib treatment and may help clinicians in a more cautious and aware management of HCC patients.

P0314

PSEUDOGENE INTS6P1 REGULATES ITS COGNATE GENE INTS6 THROUGH COMPETITIVE BINDING OF MIR-17-5P IN HEPATOCELLULAR CARCINOMA

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Background and Aims: Through complex regulation of messenger RNA (mRNA), non-coding RNA species play key roles in the pathogenesis of hepatocellular cancer (HCC). Pseudogenes are non-protein coding transcripts that display high homology with, and may act through the regulation of, their cognate protein-coding genes. However, the roles played by pseudogenes in the pathogenesis of HCC are still incompletely elucidated.
Methods: The unbiased arrays were performed to determine a putative tumor suppressive regulatory circuit composed of integrator complex subunit 6 (INTS6), integrator complex subunit 6 pseudogene 1 (INTS6P1) and miR-17-5p in HCC. A large cohort study of HCC and matched normal liver tissues is to verify the

array data. The miR-17-5p gain and loss of function were conducted to study the mediator role of miR-17-5p between INTS6 and INTS6P1. The miR-17-5p binding site in INTS6 and INTS6P1 was confirmed by luciferase assays. Functional studies included growth curves, cell death, and migration assays. In vivo studies included electroporation in a xenograft model of HCC.
Results: The large cohort study finds that INTS6 and INTS6P1 are down-regulated in a coordinated fashion in HCC. The function studies indicate that INTS6 is a novel tumor suppressor in HCC. Furthermore, mechanistic experiments indicate that the pseudogene of INTS6, namely INTS6P1, also acts as a tumor suppressor in HCC, through competition for oncomiR-17-5p.
Conclusions: Our findings demonstrate INTS6P1 and INTS6 are part of the same competitive tumor suppressor network that includes oncomiR-17-5p. This regulatory circuit reveals novel insights into the underlying mechanisms of hepatocarcinogenesis.

P0315
INCREASED AEROBIC GLYCOLYSIS IS ASSOCIATED WITH POOR OUTCOME AND SUPPRESSION OF APOPTOSIS IN HUMAN LIVER CIRRHOSIS AND HCC

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Background and Aims: Pro-inflammatory signalling in the liver promotes the appearance of a metabolic phenotype that involves the transition from mitochondrial respiration to aerobic glycolysis. It was demonstrated that this metabolic shift occurs during the transition from healthy and early stage of liver injury (NAFLD/NASH, ALD) to late stage of disease (i.e. cirrhosis), and further escalates during HCC development [1,2]. This metabolic signature enables dividing cells to satisfy anabolic and energetic needs for biomass production and to suppress apoptotic signalling, which is consistent with increased compensatory hepatic cell proliferation typical of cirrhotic and HCC livers. However other studies in contrast have suggested that hepatocytes are unable to sustain glycolysis during late stage of chronic liver disease [3].
Methods: We used unbiased gene expression analyses of microarray datasets to investigate the expression of glycolytic genes in cirrhotic and HCC livers and correlated their expression with patient outcome. Furthermore, by using a combination of *in vitro* and *in vivo* analyses we have characterized the abilities of a novel anti-apoptotic gene to regulate aerobic glycolysis in liver cirrhosis and HCC.
Results: mRNA profiling showed significantly higher expression of glycolytic transcripts in cirrhotic and HCC livers compared to normal quiescent livers ($P < 0.05$). Up regulation of *Glut1*, *Hk1*, *Hk2*, *G6PI*, and PFKL was seen in HCC livers compared to their adjacent non-tumor tissues ($P < 0.001$). Notably, expression of enzymes regulating mitochondrial activity (Pdha, Pdk) was unchanged between non-tumor tissues and late stage of HCC. Moreover, up regulation of a novel anti-apoptotic gene positively correlated with increased expression of glycolytic transcripts in a group of cirrhotic patients prospectively classified as poor prognosis based on HCC development, and promotes the aerobic glycolysis of hepatoma cells.
Conclusions: In summary, our findings delineate a putative link between aerobic glycolysis and suppression of apoptosis that is an important part of the progression of cirrhosis to HCC. The identification of the mechanism regulating this link may lead to design new therapeutic strategies for human liver disease.

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P0316
DESPITE REPRESSING IGF-1R, MIR-181A HALTS THE TUMOR-SUPPRESSOR ACTIVITY OF DECORIN BY ENHANCING TUMOR PROGRESSION IN HEPATOCELLULAR CARCINOMA

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Background and Aims: Decorin (DCN) is a tumor suppressor stromal-specific proteoglycan, and a modulator of several tyrosine-kinase receptors including insulin-like growth factor receptor 1 (IGF-1R). Thus, it was intriguing to study the effect of manipulating the expression of DCN in hepatocellular carcinoma (HCC) and consequently its effect on the oncogenic IGF-1R. Our preliminary in silico analysis predicted miR-181a to target DCN 3′ UTR with high score. The regulation of DCN by miRNAs was never investigated in HCC before. Hence we aimed at exploring this obscure relationship and its impact on IGF-1R.
Methods: Huh-7 HCC cell lines were cultured and transfected with miR-181a mimics, anti-miR-181a, and siRNAs against DCN. This was followed by total RNA extraction, reverse transcription to cDNA, and quantification with Real-time PCR. Viability and proliferation analysis were performed using MTT and BrdU assays. Unpaired t test and Pearson statistical methods were used. Data was considered statistically significant with $p < 0.05$ using GraphPad 5.0 software.
Results: Mimicking miR-181a in Huh-7 cells resulted in a significant down-regulation in DCN mRNA expression. Similarly, miR-181a mimics resulted in a concomitant significant down-regulation of IGF-1R mRNA expression. A direct correlation was observed between DCN and IGF-1R mRNAs' expression in mimicked cells. On the other hand, miR-181a was inversely correlated with both DCN and IGF-1R in miR-181a mimicked cells. Functional characterization of miR-181a in Huh-7 cells revealed induction in cellular viability and proliferation upon forcing the expression of miR-181a. Similarly, knocking down DCN gene using specific siRNAs induced Huh-7 cellular viability and proliferation.
Conclusions: The tumor suppressor activity of DCN is halted by the oncomiR miR-181a in HCC, which was observed in decreased DCN expression by miR-181a mimics. This was further proved by the induced cellular viability and proliferation by miR-181a and DCN siRNAs. On the other hand, the decrease in IGF-1R by oncomiR miR-181a remains unexplained but may be due to the direct targeting with miR-181a as predicted by our in silico analysis. Thus it can be concluded that miR-181a has a role in the interplay between DCN and IGF-1R and hence on HCC progression. These findings display a novel molecular mechanism of DCN regulation in HCC, and decreasing miR-181a levels might be a strategy for the enhancement of the tumor suppressor effects of DCN.

P0317

DOWN-REGULATION OF Hao2 IS ASSOCIATED WITH THE ONSET AND DEVELOPMENT OF HEPATOCELLULAR CARCINOMA IN RODENTS

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Background and Aims: L-2-Hydroxy acid oxidases are flavin mononucleotide (FMN)-dependent peroxisomal enzymes, which are responsible for the oxidation of a number of l-2-hydroxy acids to ketoacids at the expense of molecular oxygen, resulting in the formation of hydrogen peroxide. Its role in cancer, if any, is still unknown. The aim of this work was to investigate the expression of Hao2 in early and advanced neoplastic lesions.
Methods: qRT-PCR and western blot analysis were performed in (i) HCCs developed in mice exposed to repeated injections of the CAR ligand TCPOBOP, with or without a single administration of diethylnitrosamine (DENA); (ii) pre- and neoplastic lesions generated by the rat Resistant Hepatocyte model (RH); (iii) several human hepatoma cells.
Results: We found a strong down-regulation of Hao2 in HCCs induced in mouse liver by DENA + TCPOBOP or by TCPOBOP alone. To establish whether Hao2 down regulation occurs early in HCC development, we moved to the rat RH model of hepatocarcinogenesis which allows the identification of very early preneoplastic lesions. Using this model, we found that both mRNA and protein expression of Hao2 were profoundly down-regulated in early preneoplastic lesions. Down regulation of Hao2 was maintained all throughout the process (early and advanced HCCs). An almost undetectable Hao2 expression was observed also in 5 different human hepatoma cell lines. Interestingly, down-regulation of Hao2 was associated with miRNA-183 and miR-223, which are predicted to target Hao2 mRNA.
Conclusions: These results describe, for the first time, the dysregulation of a metabolic gene whose expression is severely impaired in HCC generated in two different species and by different etiological agents. They also demonstrate that dysregulation of Hao2 is a very early event in the development of HCC, and suggest that Hao2 expression is under the control of two miRNAs overexpressed in all the stages of hepatocarcinogenesis.

P0318

ULTRA-DEEP SEQUENCING OF MULTIPLEX-PCR ENRICHED HOTSPOT AND DISEASE-RELATED TARGETS IN HEPATOCELLULAR CARCINOMA

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Background and Aims: Point mutations and small insertions/deletions (indels) in tumor relevant genes have been identified and proposed as new indicators in hepatocellular carcinoma prognosis. Next generation sequencing (NGS) technologies provide the simultaneous analysis of genomic mutations in numerous target regions with high sensitivity. In the present study, based on data by whole exome analysis, a hotspot targeted multiplex PCR approach was combined with ultra deep sequencing and applied to hepatocellular carcinoma representing different grade of progression.
Methods: 72 HCC nodules of different etiology and grades were macrodissected and DNA extracted. At least 3000 genomic copies were amplified by multiplex PCR using primer sets targeting a wide panel of hotspot cancer genes. 394 amplicons of each sample were generated and sequenced by means of the MiSeq platform (Illumina). Conventional Sanger sequencing served as a reference technology. Alignment and variant calling was performed using the CLC Cancer Workbench software (CLC Bio, QIAGEN).
Results: Each macrodissected HCC nodule was sequenced by more than one million reads covering each targeted hotspot locus between approximately 400 and 2,000 reads. Variants were detected in exons of HNF1a, PTEN, retinoblastoma, Axin1, CTNNB1, CDH1, SMAD4 and APC. Variants of ARID2, and ARID1A were affected in high frequency by mutations. Interestingly, different nodules of one liver biopsy showed mostly but not always similar variant pattern, but in different extent as we expected from histological classification of the hepatocellular dedifferentiation.
Conclusions: We established a fast pipeline of mutation analysis of a tumor specific, hotspot gene panel covering diagnostic relevant loci of HCC by 500–2000 reads. For prognostic and therapy relevant data interpretation numerous nodules should be sequenced to get a representative mutation status for further clincial treatments.

P0319

IDENTIFICATION OF MOLECULAR ALTERATIONS INDUCED BY TARGETING METABOLISM IN HEPATOCELLULAR CARCINOMA

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Background and Aims: Cancer cells significantly rely on energy-rich substrates, e.g. glucose, for survival, proliferation and invasiveness. However, studies suggest that tumours can evade cell death by switching metabolic pathways or substrates when challenged by microenvironmental insults. Identification of regulators of these metabolic alterations will potentially guide targeted cancer therapy with an attendant improved prognosis. In this study, we aimed to identify signalling and genomic changes in hepatocellular carcinoma (HCC) following treatment with anti-metabolic drugs.
Methods: A panel of HCC cells, representative of early and advanced stages, were treated with anti-metabolic drugs, including 2 deoxy glucose (2-DG), metformin and also transforming growth factor beta 1 (TGF-β1). Functional effects were evaluated by cell proliferation, ATP production, clonogenicity, migration, caspase-3 assay and flow cytometry. Signalling alterations were studied using immunoblotting techniques and differential gene expression was analysed by quantitative real-time PCR of selected potential target genes. The target genes were predicted by in silico analyses of microarray datasets available in public domains.
Results: We find that HCC models harbour divergent metabolic portraits, and show varying degree of sensitivity to metabolism targeting drugs. Accordingly, HCC cells with epithelial phenotype (e.g. HUH7) were more responsive to metformin, and mesenchymal-like cell types (e.g. HLE) to 2-DG. In combination, both drugs significantly reduced proliferation, ATP production, colony formation and absolute cell count in HLE and HUH7, with >3 fold induction of caspase-3 in HLE cells. Further analyses implicated pro-survival pathways: while ERK1/2 phosphorylation was detected in both cell types, only HUH7 had obvious loss of pERK1/2 upon combinatorial treatment. Increased phospho-AKT was also observed only in HUH7 cells. This was, however, contrary to TGF-β induced cell death in HUH7 in which total AKT but not pAKT was increased. Finally, CAV1, implicated in tumour progression was strongly downregulated in HLE by 2-DG treatment, while other genes involved in metabolic alterations, e.g. SLC16A1, were differentially regulated upon targeting metabolism.
Conclusions: We report that divergent stage-dependent molecular alterations are consequent upon targeting metabolism in HCC. A mechanistic study of implicated genes and signalling pathways could help in defining optimal therapeutic options for patients with various HCC grades.

P0320

TARGETING TGF-BETA I WITH THE TRANSFORMING GROWTH FACTOR RECEPTOR TYPE I KINASE INHIBITOR, LY2157299, MODULATES STEMNESS-RELATED BIOMARKERS IN HEPATOCELLULAR CARCINOMA

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Background and Aims: The stemness of Hepatocellular Carcinoma (HCC) is likely implicated in cancer heterogeneity, recurrence and therapy resistance. Aim of this study is to investigate the effects of TGF-beta and its type I receptor kinase inhibitor, LY2157299, on stemness-related biomarkers.
Methods: We investigated, by qRT-PCR and flow cytometry, the expression levels of EpCAM, CD133, CD13, Keratin-19 (CK-19), CD44, CD-133 and CD90 in early and late TGF-beta signature cells, after 48 hours of treatment with TGF-beta or LY2157299, respectively. Human tissue samples from patients who underwent surgery were cultured for 48 hours in the presence of LY2157299 and processed by qRT-PCR.
Results: In the early TGF-beta signature cells, the stem cell biomarkers EpCAM, CD44, CD90, CD133 and CK-19 were less expressed than in the late TGF-beta signature cells. However, TGF-beta stimulation upregulates their expression in early TGF-β signature cells, while LY2157299 downregulates their expression in late TGF-β signature cells. Consistently with the mRNA data, at the protein level TGF-beta treatment increases, whereas LY2157299 decreases, the expression of EPCAM, CD133 and CK-19 in early TGF-beta cell lines. Interestingly, the early TGF-beta signature cells do not express CD90 at basal level nor after TGF-beta treatment. However, downstream TGF-beta signalling molecules (Fibronectin, MAPK) are activated in these cell lines, indicating that TGF-beta signalling is switched on. In the late TGF-beta signature cell lines, the stimulation of TGF-beta increases the expression of CD-90 as well as activating *MAPK* and Fibronectin. In ex-vivo experiments, the CD-90 and CK-19 results paralleled those observed in HCC cell lines treated with LY2157299. A higher expression of EPCAM, AFP, CD44 and CD90 indicated an aggressive tumor pattern.
Conclusions: Inhibition of the TGF-beta pathway leads to a modulation of stemness-related biomarkers in HCC cell lines and in human HCC tissue samples.

P0321

INCREASED ANTI-TUMOR EFFECT OF VITAMIN D AFTER CYP24A1 INHIBITION ON HCC CELL LINES

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Background and Aims: 24-hydroxylase (CYP24A1) is the main inactivating enzyme of 1alpha,25-dihydroxyvitamin D3 (1,25-D3). The anti-tumor effect of 1,25-D3 is well documented. Increased expression of CYP24A1 mRNA after vitamin-D3 administration has been shown in hepatocellular carcinoma (HCC) cell lines. We investigated the change of antiproliferative effect of 1,25-D3 after administration of CYP24A1 inhibitors.
Methods: 1,25-D3 and 16 newly synthetized tetralone compounds as CYP24A1 inhibitors were simultaneously added to the HepG2 and Hep3B human HCC cell lines. Cell proliferation was measured by bromodeoxyuridine (BrdU colorimetric) incorporation and the cytotoxicity by lactate dehydrogenase (LDH) detection in the cell culture supernatant. The CYP24A1 mRNA expression was measured by real time reverse transcription-polymerase chain reaction (RT-PCR).
Results: The CYP24A1 expression reached the peak value at 8 hours after 1,25-D3 administration and returned to the almost initial range at 16 hours in HepG2 cells. There was no measurable CYP24A1 expression in Hep3B cell line. Only two inhibitor compounds (KD-35 and BB-8) out of 16 reduced significantly the cell proliferation in the presence of 1,25-D3 in HepG2 cell line compared to the control cells. In Hep3B cells there was no significant change in proliferation. The cell proliferation reduction was 53% in the presence of BB-8 and 30% with of KD-35 after 32 hours incubation with 100 nmol/L 1,25-D3 and 5 μmol/L CYP24A1 inhibitors compared to the control cells treated only with 1,23-D3 (100 nmol/L). Neither BB-8 nor KD-35 alone or co-administered with 1,25-D3 had direct cytotoxic effect based on the LDH levels.
Conclusions: Our novel data show increased antitumor effect of 1,25-D3 on human HCC cell line after co-administration with CYP24A1 inhibitors. The BB-8 showed the highest inhibitory potential on CYP24A1 in HepG2 cells. The use of CYP24A1 inhibitors might decrease the dose of 1,25-D3 in the antitumor treatment in the future.

P0322

GLYPICAN-3 PROMOTES HEPATOCELLULAR CARCINOMA PROGRESSION AND METASTASIS BY ACTIVATION OF THE ERK SIGNALING PATHWAY

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Background and Aims: Glypican-3 (GPC3), a membrane-associated heparan sulfate proteoglycan, is frequently up-regulated in hepatocellular carcinoma (HCC). However, how GPC3 contributes to progress of HCC is largely unclear. The present study investigated the association between GPC3 expression and HCC clinicopathological characteristics, and particularly focused on the role and underlying mechanisms of GPC3 in HCC epithelial–mesenchymal transition (EMT).
Methods: Expression of GPC3 in paired liver tissues (tumor versus non-tumor) was examined in 45 patients with HCC by quantitative real-time PCR, immunohistochemistry, and western blotting, respectively. Correlations between GPC3 expression, clinicopathogenical features and EMT-related biomarkers were analyzed in these patients. In vitro, the effects of GPC3 on cell migration, invasion and EMT were investigated in 3 HCC cell lines (HepG2, Hep3B and Huh 7) by scratch, transwell assays and Western blot analyses.
Results: In comparison with surrounding non-tumor tissue, remarkably elevated expression of GPC3 demonstrated in HCC tumor tissues. The tissue expression of GPC3 was increased during HCC progression from BCLC stage A or B to stage C. The enhanced levels of GPC3 in HCC tumor tissues were tightly correlated to expression of the EMT-associated proteins and tumor vascular invasion. Moreover, patients with GPC3-high expression in tumor tissues displayed significantly shorter survival time than those with GPC3-low expression ($P = 0.041$). Consistent with the findings in patients, HepG2 cells, which expressed high levels of GPC3, showed stronger capacity of migration and significant EMT-like changes when compared to those HCC cells with low levels of GPC3, e.g., Hep3B

and Huh7. Furthermore, administration with exogenous GPC3 in HCC cells activated extracellular signal-regulated kinase (ERK) and significantly enhanced cell migration and invasion. These behaviors were significantly inhibited by an ERK inhibitor, PD98059.
Conclusions: GCP3 contributes to HCC progression and metastasis through impacting EMT of cancer cells. The effects of GCP3 are associated with ERK activation.

P0323
LDH SERUM LEVELS AS PROGNOSTIC AND PREDICTIVE FACTOR IN ADVANCED BILIARY TRACT CANCER PATIENTS TREATED WITH FIRST LINE CHEMOTHERAPY

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Background and Aims: Previous data suggested that LDH serum levels may be associated with tumour hypoxia and VEGFA and VEGFR-1 over-expression. LDH may then represent an indirect marker of activated tumour neo-angiogenesis and worse prognosis in many tumour types. In our analysis, we analyzed the role of LDH serum levels in predicting clinical outcome for biliary tract cancer patients treated with first-line cisplatin and gemcitabine chemotherapy, to individuate a potentially reliable and easy to use marker for patients stratification.
Methods: 71 advanced biliary tract cancer patients treated with cisplatin and gemcitabine in first-line chemotherapy were available for our analysis. For all patients, LDH values were collected within one month before treatment beginning. We chose the laboratory cut-off (Upper Normal Rate, UNR) as LDH cut-off value (450 U/l) and then we divided the patients into two groups (A and B, below and above the UNR respectively). Survival distribution was estimated by the Kaplan–Meier method. Disease control rate (DCR) was assessed with chi-square test. A significant level of 0.05 was chosen to assess the statistical significance.
Results: Patients in group A (46 patients) and B (25 patients) proved homogeneous for all clinical characteristics analyzed. Median progression free survival (PFS) was 3.97 months and 1.8 months respectively in group A (patients with LDH level below the UNR) and in group B (patients with LDH level above the UNR), $p = 0.0064$ (HR = 2.07, 95% CI: 1.07–3.99). Median overall survival (OS) was 9.24 months and 2.55 months in group A and B respectively, $p < 0.0001$ (HR = 2.93; 95% CI: 1.37–6.27). DCR was 65% in group A vs. 21% in group B ($p = 0.004$).
Conclusions: Our observations seem to suggest a prognostic role of LDH in biliary tract cancer patients. Our findings showing an improved PFS and DCR in patients with low LDH serum levels also suggest a possible predictive role in patients treated with a cisplatin and gemcitabine regimen as first-line chemotherapy. After further confirmation in larger trial, these results may be relevant for a better patients stratification and selection.

P0324
EpCAM CLEAVED EXTRACELLULAR DOMAIN HAS PROFOUND EFFECT ON HUMAN PROGENITOR CELL LINE HepaRG, BUT BARELY EFFECTS HEPATOCARCINOMA CELL LINE HepG2

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Background and Aims: EpCAM has long been established as an important marker of liver cancer cells and a prognostic predictor. Its signalling activities in cancer development have been studied in some detail, exposing an ambiguous role in tumour cell proliferation and metastasis, but little is known about the profile of EpCAM-responsive cells. EpCAM anchored to the cell membrane mediates homophilic adhesion interactions and cell-to-cell signalling. The extracellular domain of EpCAM (EpEx) can also be cleaved off by various proteolytic enzymes, releasing a molecule with proposed cytokine-like functions. We set out to study the effect of EpEx on human hepatic progenitor and tumour cell lines.
Methods: cDNA encoding EpEX (243 aa) together with the signal peptide for secretion was obtained via RT-PCR from the human hepatocarcinoma cell line Huh7.5 and together with EGFP as a marker gene, cloned into the mammalian expression vector pCDNA3.1. HEK-293T cells were transfected with the vector. After verification of EpEx-secretion, the EpEx expressing cells were cocultured with the human hepatic progenitor cell line HepaRG, or the human hepatocarcinoma cell line HepG2. Following a 10 day long incubation protocol their gene expression profile was compared to cells co-cultured with EGFP-only expressing HEK-293Ts.
An RT-qPCR panel of more than 120 transcripts indicative for liver cell differentiation and maturation status was employed in the experiment analysis.
Results: In HepaRG cells, the expression of the transcription factor Onecut and the receptor Notch 3 have been greatly upregulated (50- and 10-fold, respectively), while members of the Wnt/β-catenin pathway for instance were not affected. Cytokeratins 7 and 14 also showed a 15–18 fold increase in expression, and elevated levels of SALL4, SOX17, TAT, CD34, Gys2, Patched1, Notch1 and GGT were observed. On the other hand, EpEx effects on HepG2 cells were not significant.
Conclusions: While both the HepG2 and HepaRG cell lines are EpCAM positive themselves to a similar extent, only the latter is responsive to EpEx signalling. The mechanisms of the responses observed need to be further elucidated, but are likely to be connected to the progenitor specific characteristics of HepaRGs. Amongst other findings to be presented, the results hint towards a possible cross talk between EpEx and the Notch cascade.

Liver tumors: b. Clinical (epidemiology, diagnosis)

P0325
EVERY 3-MONTH SURVEILLANCE IS SUPERIOR TO SEMIANNUAL SURVEILLANCE FOR THE SURVIVAL OF PATIENTS WITH HEPATOCELLULAR CARCINOMA AFTER HEPATECTOMY: A 10-YEAR OBSERVATIONAL STUDY

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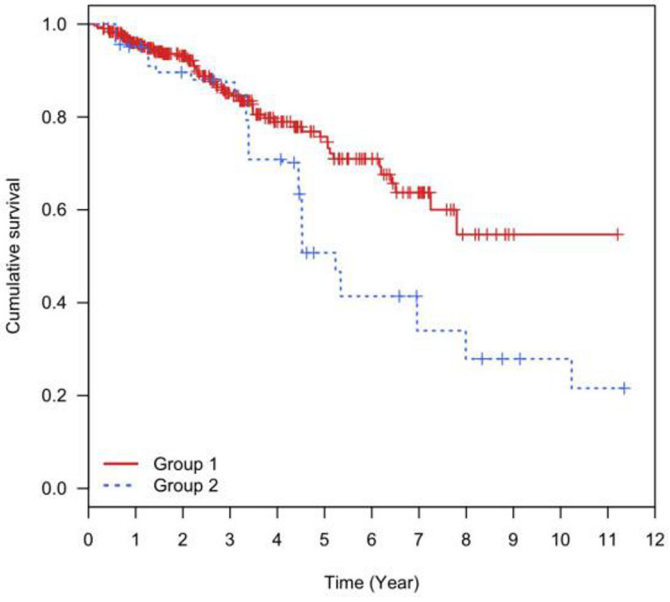
Background and Aims: The current guidelines recommend the surveillance of patients treated with curative hepatectomy for hepatocellular carcinoma (HCC) recurrence, based on liver computed tomography (CT) repetition at 3 month intervals. Since there is no compelling evidence of superiority of the stringent program, this study aimed at comparing survival rates between

patients treated with hepatectomy for very early or early stage HCC in Barcelona Clinic Liver Cancer (BCLC) stage on every 3-month and semiannual surveillance.

Methods: This retrospective study included 456 prospectively registered consecutive patients treated with hepatectomy for HCC. Patients were divided into two groups according to surveillance intervals; 307 patients submitted to every 3-month (Group 1) and 35 patients to semiannual surveillance (Group 2). Patients without recurrence in 2 years after hepatectomy were divided into two surveillance groups; 88 patients in every 3-month (Group 3) and 114 patients who underwent every 3-month surveillance until 2 years and changed to semiannual surveillance after 2 years (Group 4). The survival rates comparing each group were analyzed using inverse probability weighting according to liver cirrhosis or HCC risk factors. The results were presented as corrected for the lead time bias. Univariate and multivariate analyses were performed to identify risk factors for recurrence.

Results: The median follow-up duration was 30, 53, 40, and 72 months in Group 1, 2, 3, and 4, respectively. The survival rates in Group 1 was significantly higher than those in Group 2; hazard ratio of 1.83 ($P < 0.01$). In patients without HCC recurrence in 2 years, the survival rates in Group 3 were not significantly different from those in Group 4 ($P = 0.12$). Alpha-fetoprotein (AFP) and microvascular invasion (MVI) were independent prognostic factors for HCC recurrence in 2 years. Patients with high AFP level (≥50) and/or MVI showed poorer prognosis than those with low AFP level (<50) and no MVI in Group 1 and 2 ($P < 0.05$).

Conclusions: Every 3-month surveillance improves the survival of patients treated with hepatectomy for HCC in BCLC stage 0 or A as compared to the semiannual program. However, there were no benefits of every 3-month surveillance over semiannual surveillance after 2 years in patients without HCC recurrence in 2 years after treatment. Posthepatectomy surveillance CT schedules may be optimized according to risk stratification for recurrence to reduce the frequency of CT scans without compromising surveillance benefits.



P0326

HEPATOCELLULAR CARCINOMA (HCC) IN HCV-PATIENTS WITH OR WITHOUT HIV-COINFECTION. SIMILAR SURVIVAL IN A NATIONWIDE STUDY

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Background and Aims: HCV end-stage liver diseases including liver decompensation (LD) and HCC represent the first cause of mortality in coinfected HIV-HCV patients. Published data on the influence of HIV on the prognosis of HCC are controversial. The aim of this study was to address this issue in an all-inclusive, nationwide analysis, taking into account potential confounders.

Methods: The 2007 to 2012 National French health care databases were screened to identify all hospital admission that included a diagnostic code of HCC and HCV. Incident cases of HCC were selected by defining the entry point as a first admission in 2009. Demographic data, associated conditions, underlying liver disease, as well as the type, location and annual HCC-caseload of the hospital were retrieved. Treatments were stratified into curative (transplantation, surgical resection, trans-dermal ablation), palliative or others. The characteristics and survival of HIV+ and HIV− HCV-patients with HCC were compared.

Results: Between 2009–2012, 5,067 incident-cases of HCC in HCV-patients were identified including 268 (5.3%) in HIV+ patients. HIV+ patients were significantly younger (51.9±6.5 vs. 63.9±12.8 years), had fewer comorbidities (diabetes, obesity, arterial hypertension) and more frequently co-étiologies associated with HCV. The frequency of LD and metastatic form at diagnosis were similar. The type of treatment received was not different and curative treatment was realized in 35.4%. HIV+ patients were more frequently followed in high-volume structures and in teaching hospitals, and significant geographic regional differences were observed. In multivariate analysis, only age, co-etiologies and geographic region remained significant. The crude survival was not different in HIV+ and HIV− patients (15.2 [9.4–22.4] vs. 18.0 months [16.5–19.3]) and was influenced by age, the presence of cirrhosis and LD at diagnosis, the presence of co-morbidities (diabetes, arterial hypertension), the hospital case-load and the type of treatment. After adjusting for confounders, the survival remained not different in HIV+ and HIV− patients (16.3 [11.6–22.4] vs. 18.4 months [17.4–19.2]).

Conclusions: In this nationwide study of HCC in HCV-patients, the adjusted survival is not different in HIV+ and HIV− patients. Despite of a relatively high rate of curative treatment, the prognosis remains poor and underlines the need for early anti-HCV treatment in both HIV+ and HIV− patients

P0327

ANALYSIS OF HEPATIC PROGENITOR CELL MARKER POSITIVE HEPATOCELLULAR CARCINOMA AND THEIR PROGNOSTIC SIGNIFICANCE

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Background and Aims: Much attention has been paid to hepatic progenitor cell (HPC) marker-positive hepatocellular carcinoma (HCC) as a marker of poor prognosis of patients with HCC. Cytokeratin19 (CK19), neural cell adhesion molecule (NCAM), and epithelial cell adhesion molecule (EpCAM) are known HPC markers on HCC. However, the relationship between each HCC marker, the

characteristics of each marker, and the relationship between HPC marker-positive HCC and serum levels of previously reported tumor markers such as AFP, as well as the frequency of fucosylated-AFP (AFP-L3) and des-γ-carboxy prothrombin (DCP) remain unclear. In this study, we analyzed 202 HCC nodules to elucidate the prognostic significance of each HPC marker-positive HCC.

Methods: Surgically removed HCC were stained for CK19, NCAM and EpCAM. Expression of each HPC marker was considered positive if more than 5% of tumor cells were stained. The frequency of each marker single-, double-, and triple-positive HCC, the recurrence free survival (RFS), and overall survival (OS) were calculated and the relationship between these serum markers and HPC marker-positive cells was analyzed.

Results: The frequency of each HPC marker-positive HCC was as follows: $CK19^+NCAM^-EpCAM^-$ (6 cases, 3.0%), $CK19^-NCAM^+EpCAM^-$ (11 cases, 5.4%), $CK19^-NCAM^-EpCAM^+$ (15 cases, 7.4%), $CK19^+NCAM^+EpCAM^-$ (1 case, 0.5%), $CK19^+NCAM^-EpCAM^+$ (8 cases, 4%), $CK19^-NCAM^+EpCAM^+$ (2 cases, 1.0%), $CK19^+NCAM^+EpCAM^+$ (1 case, 0.5%), and $CK19^-NCAM^-EpCAM^-$ (158 cases, 78.2%). CK19 and EpCAM are often co-expressed, this double-positive group showed particularly high serum levels of AFP and AFP-L3. In contrast, $NCAM^+$ HCC differed from CK19 and EpCAM HCC and showed relatively low serum levels of AFP and AFP-L3, and relatively high serum levels of DCP. No significant difference in the RFS between each marker-positive HCC was observed. However, the OS did differ significantly: EpCAM has poorest prognosis, followed by NCAM and CK19 has the best prognosis. Importantly, CK19 single-positive HCCs exhibited significantly better prognosis than CK19 and EpCAM double-positive HCCs, indicating that EpCAM-positive cells in the CK19-positive HCC population plays a major role in the poor prognosis of $CK19^+$ HCC.

Conclusions: EpCAM is a better predictor of prognosis than CK19. NCAM is a strong indicator of poor prognosis and marked different populations to CK19 and EpCAM. We consider that EpCAM and NCAM, in addition to serum levels of tumor markers, are useful in predicting the prognosis of HCC patients.

P0328

INCIDENCE, CHARACTERISTICS AND DETERMINANTS OF PRIMARY LIVER CANCER OCCURRING DURING SURVEILLANCE OF COMPENSATED HCV CIRRHOSIS ACCORDING TO SUSTAINED VIROLOGICAL RESPONSE (ANRS CO12 CirVir)

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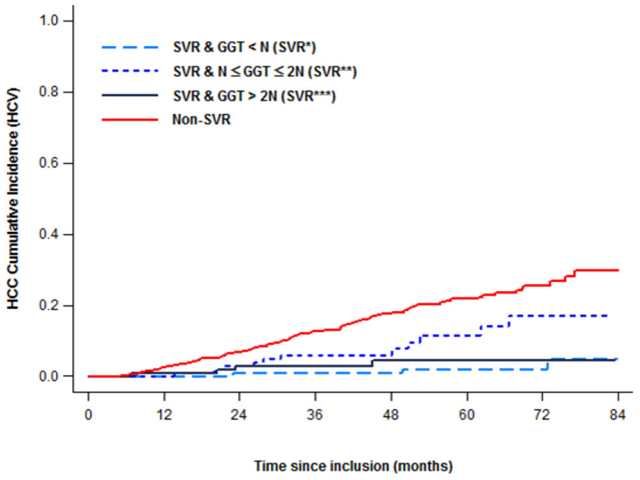
Background and Aims: The objective was to prospectively assess the benefits of sustained viral response (SVR) on the incidence of primary liver cancer (PLC) in French patients with compensated HCV cirrhosis and to describe their characteristics.

Methods: Patients with the following inclusion criteria were included in 35 French centres: (a) biopsy-proven HCV cirrhosis; (b) Child–Pugh A; (c) absence of previous liver complications. Patients were prospectively followed-up every 6 months.

Results: A total of 1323 patients were enrolled between March 2006 and June 2012 [age 55, men 63.5%, Genotype 1: 62%, 2: 5%, 3: 14%, 4: 8%, MD: 11%]. At end-point in January 2014 (median follow-up of 43 months), SVR was observed in 458 (34.6%) patients after at least one interferon-based regimen. PLC was diagnosed in 141 [136 hepatocellular carcinoma (HCC) and 5 cholangiocarcinomas]. SVR was an independent factor associated with a decreased incidence of HCC (Cumulative incidence, CumI 5 years: 5.1% vs 20%, HR = 0.35 [0.20–0.62], $P < 0.001$). In patients with SVR, 18 HCC were diagnosed. At diagnosis, as compared to patients without SVR, the rate of HCC within Milan criteria was higher in SVR patients (88.9% vs 81.1%, $P = 0.7$), median serum AFP level was lower (4 ng/mL [3–7.4] vs 20 [9.7–101], $P < 0.0001$), and the rate of first-line curative treatment was higher (83% vs 77%, $P = 0.7$). The last imaging examination was performed 5.9 [5.4–6.6] months before HCC diagnosis in the SVR group, compared to 6.7 [5.7–8.1] ($P = 0.07$) in the non-SVR group. In multivariate analysis in SVR patients, only lower PT <80% (HR = 4.97 [1.93–12.84], $P = 0.001$) and higher GGT levels >ULN (HR = 4.30 [1.24–14.90, $P = 0.006$) were independent HCC risk factors, while in non SVR patients HCC predictors were age >50, past excessive alcohol intake, higher GGT levels and lower platelet count. The overall mortality was lower in SVR patients (CumI 5 years 3.8% vs 15.3%, log-rank <0.0001). Among the 16 deaths recorded in SVR patients, progression of HCC (13.3%) was the fourth cause of death after end-stage liver disease (33.3%), extra-hepatic cancers (26.7%) and other extra-hepatic diseases including bacterial infection (25%).

Conclusions: A four-fold reduction in HCC incidence is observed in patients with HCV compensated cirrhosis achieving SVR. HCC developed after SVR could be less advanced at diagnosis than tumours occurring in the absence of SVR, even if this result needs confirmation.



	At-risk patients (events)														
SVR*	185	(0)	170	(1)	143	(0)	124	(0)	94	(1)	73	(0)	37	(1)	2
SVR**	137	(0)	126	(3)	102	(3)	75	(0)	53	(3)	37	(2)	15	(0)	2
SVR***	115	(1)	107	(2)	91	(0)	73	(1)	57	(0)	36	(0)	20	(0)	2
Non-SVR	741	(16)	649	(28)	527	(30)	392	(19)	269	(11)	171	(6)	80	(3)	9

P0329

THE ITA.LI.CA STAGING SYSTEM FOR PATIENTS WITH HEPATOCELLULAR CARCINOMA: A MULTICENTER COHORT STUDY

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Background and Aims: The Barcelona Clinic Liver Cancer (BCLC) classification stratifies patients with hepatocellular carcinoma (HCC) in different evolutionary stages. While it showed to help treatment allocation especially in the context of clinical trials, its prognostic ability to stratify HCC patient survival still remains controversial when compared to that of other systems (HKLC, MESIAH, CLIP, JIS). The aim of this study is to develop and validate a new staging system for HCC patients taking as a reference the original BCLC evolutionary stages but focused in optimizing individual patient survival prediction.
Methods: The ITA.LI.CA (Italian Liver Cancer) database (n = 5183, period 1986–2012) was divided in training (n = 3628, 70%) and validation (n = 1555, 30%) cohorts. A further cohort of 2651 HCC patients from Taiwan was used for external validation. We defined the ITA.LI.CA Tumor Status (TS) using BCLC stages (0, A, B, C) but adding 3 intermediate B sub-stages (based on TNM and HKLC staging): B1 = single >5 cm or 2–3 nodules 3–5 cm; B2 = 2–3 nodules >5 cm or >3 nodules ≤5 cm; B3 = >3 nodules >5 cm or presence of intrahepatic macrovascular invasion. TS was then included in a multivariate survival model together with Child–Pugh Score (CPS) and ECOG performance status (PS), and a novel ITA.LI.CA prognostic staging was developed. Discrimination and calibration of the new prognostic model was internally and externally validated and compared with that of other staging systems.

Variables	0n	An		B1n		B2n		B3n		Cn
Diameter(cm)	< 2	≤ 3	≤ 5	3-5	> 5	3-5	> 5	> 5	Any	Any
N° nodules	1	2-3	1	2-3	1	> 3	2-3	> 3	Any	Any
Vascular invasion or metastases	no	no	no	no	no	no	no	no	Intrahep.	Extrahep./ Meta

n score based on PS and CPS

0: PS 0 and CPS 5 → best possible HCC therapy

1: PS 1 - 2 or CPS 6 -7 → relative controindication to HCC therapy

≥ 2: PS 1-2 and CPS 6-7 or PS > 2 or CPS> 7 → absolute contraindication to HCC therapy

Figure: The ITA.LI.CA staging system for HCC patients.

Results: The ITA.LI.CA staging system was based on the following stages: 0n, An, B1n, B2n, B3n, Cn, where capital letters defined TS stages indicating potential for treatment as in BCLC (from radical to palliative therapies), while n defined an additional score (0/1 = eligible for HCC therapy; 2/>2 = contraindication to therapy) based on CPS and PST indicating treatment feasibility. Discrimination ability of the ITA.LI.CA staging system was higher than that of the other systems in the training, internal, and external validation cohorts. The concordance (c)-statistics for this model in training, internal and external validation cohorts were respectively 0.72, 0.71, and 0.78, and they were superior to that for BCLC (0.65, 0.64, and 0.73), CLIP (0.69, 0.68, and 0.75), JIS (0.67, 0.67, and 0.70), MESIAH (0.69, 0.69, and 0.77), and HKLC (0.68, 0.68, and 0.75).

Conclusions: We developed a novel ITA.LI.CA staging system, easy to apply in clinical practice, and with a strong prognostic impact in two large western and eastern populations.

P0330

COST-EFFECTIVENESS ANALYSIS OF HEPATOCELLULAR CARCINOMA SURVEILLANCE IN PATIENTS WITH HEPATITIS C RELATED CIRRHOSIS AFTER SUSTAINED VIROLOGICAL RESPONSE

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Background and Aims: Hepatitis C virus (HCV) causes hepatocellular carcinoma (HCC) in patients with cirrhosis. New therapies eradicating HCV-infection lead to sustained virological response (SVR). Screening for HCC in cirrhosis patients, as recommended by guidelines, is cost-effective. However, since SVR substantially decreases the risk for HCC, cost-effectiveness of screening for HCC in these patients is unknown. Therefore, our objective is to evaluate cost-effectiveness of biannual ultrasound (US) HCC surveillance in HCV-related cirrhosis patients post-SVR.
Methods: We designed a Markov state transition model to simulate the natural history of HCV post-SVR. A lifetime time horizon was used in a cohort of 50-year-old cirrhosis patients post-SVR to evaluate biannual US screening and management of HCC by screen-all vs. screen-none strategies. Based on AASLD guidelines, tumors detected by US were confirmed by additional dynamic imaging techniques. Parameter values including probabilities, utilities and costs were obtained from the literature and if not available, experts' opinions were sought. Costs were in CAN$, health outcomes were measured as quality adjusted life years (QALYs) and both were discounted at 5%. Sensitivity analyses were conducted to assess parameter uncertainty.
Results: With 0.5% HCC annual incidence rate in the model population, biannual US screening offered a gain of 0.096 QALYs vs. no screening through early tumor detection and proper management. Liver-related mortality was decreased by about 20%. The calculated costs were $41,475 in screen-all and $27,625 in screen-none strategies, resulting in an incremental cost-effectiveness ratio of 149,590/QALY. Sensitivity analysis on model variables demonstrated that the results are most sensitive to annual discount rate, HCC incidence rate, asymptomatic HCC to symptomatic tumor transition probability and also utility and cost of living without HCC. Probabilistic sensitivity analysis showed that with $50,000/QALY as willingness to pay threshold, in 98% of cases the screen all strategy would not be cost-effective. This trend starts to reverse if the threshold is $150,000/QALY and over.
Conclusions: We found that with 0.5% HCC incidence in HCV-related cirrhosis patients post-SVR and the cost-effectiveness threshold of $50,000/QALY, this strategy would not be cost-effective. However, we need to find better ways to identify patients with high risk for HCC post-SVR because HCC surveillance for all patients with cirrhosis is likely not cost effective.

P0331

THE 'GALAD SCORE' FOR SEROLOGICAL DETECTION OF HEPATOCELLULAR CARCINOMA: INTERNATIONAL VALIDATION AND ASSESSMENT OF THE INFLUENCE OF TUMOUR SIZE AND AETIOLOGY ON MODEL UTILITY

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Background and Aims: A statistical model that uses objective measures to estimate the likelihood that hepatocellular carcinoma (HCC) is present in patients with chronic liver disease (CLD) has recently been developed. This model, GALAD, uses Gender, Age and the serological tumor markers AFP-L3, AFP and DCP. It has the potential to be used in the screening/surveillance setting, has not been validated in an international setting.

Methods: Cohorts comprising 4476 patients from Japan (1514 HCCs and 2962 CLDs) and 1086 from Germany (238 HCCs and 848 CLDs) were recruited. In each case sera and related clinical features were collected by investigators independent of the laboratory in which the biomarker assays were performed and the group performing the statistical analysis. We also included, for reference, the original UK cohort on which the model was developed (394 HCCs and 439 CLDs). We assessed the change in sensitivity, specificity and area under the ROC curve (AUROC) when the new model was compared to the conventional approach to using these biomarkers in the Japanese screening program (Table). We also assessed the impact of tumour size and aetiology on model performance.

Table: Sensitivity, specificity, and area under the curve (AUROC) for the biomarkers and the GALAD model

	Cut-off [a]	Sensitivity (%)	Specificity (%)	AUROC
Japan				
AFP	20 ng/mL	51.3	97.3	0.89
AFP-L3	7%	41.2	91.8	0.75
DCP	0.48 ng/mL	57.3	97.4	0.84
AFP + AFP-L3 + DCP	Same as above	79.3	88.3	0.84
GALAD Model	−1.55	82.2	87.6	0.93
Germany				
AFP	20 ng/mL	57.6	93.2	0.87
AFP-L3	7%	70.2	79.0	0.83
DCP	0.48 ng/mL	88.2	67.1	0.87
AFP + AFP-L3 + DCP	Same as above	94.5	56.1	0.75
GALAD Model	−0.63	86.6	89.0	0.95
United Kingdom				
AFP	20 ng/mL	60.7	96.4	0.88
AFP-L3	7%	75.4	73.5	0.84
DCP	0.48 ng/mL	94.0	63.7	0.90
AFP + AFP-L3 + DCP	Same as above	99.2	50.0	0.75
GALAD Model	−0.63	91.6	89.7	0.97

[a] Cut-off points for three biomarkers were based on the guideline of the Japan Society of Hepatology and our previous studies.

Results: In the Japanese and German cohorts, AUROC was 0.93 and 0.95 respectively, both almost identical to the results in the UK series (0.97). The model showed better diagnostic accuracy than when the biomarkers were used individually (Table). Additionally the model utility increased slightly in larger tumours (≥5 cm) with an AUROC of 0.98, 0.97 and 0.98 in the Japanese, German and UK cohorts respectively. Aetiology had no impact on model performance in the UK and German cohorts.

Conclusions: The GALAD model for serological diagnosis of HCC has been validated by application to large patient cohorts from Germany and Japan.

P0332

GENE ALTERATIONS IN TERT PROMOTER, CTNNB1, AND TP53 ARE CLOSELY ASSOCIATED WITH DEVELOPMENT AND PROGNOSIS OF HEPATOCELLULAR CARCINOMA: COMPREHENSIVE ANALYSES BY NEXT GENERATION SEQUENCING TECHNOLOGY

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Background and Aims: Genetic alterations in specific genes lead to disruption of cellular pathways and are thought to be critical events in the hepatocarcinogenesis and progression of hepatocellular carcinoma (HCC). However, such genetic alterations responsible for tumor development and progression are still unclear. To identify genetic alterations associated with hepatocarcinogenesis and prognosis of HCC, we comprehensively investigated genetic alterations in paired HCC and surrounding non-HCC tissues using next generation deep sequencing technology.

Methods: The exacted DNA from 104 patients was amplified by multiplexed polymerase chain reaction (PCR) targeting 50 cancer-related genes containing 2790 mutational hotspots. The mutations were analyzed using a semiconductor-based DNA sequencer. Alignment to the hg19 human reference genome and variant calling was carried out, and nucleotide variants that were detected in tumors and absent in corresponding non-tumor tissue were determined as somatic mutations. We also investigated TERT promoter mutation at 2 hot spots through direct sequencing. Association between gene mutation and data on clinical outcomes were analyzed.

Results: The mean coverage depth was 2088 per sample. We found that TERT, TP53, CTNNB1, PTEN, CDKN2A, HRAS, PIK3CA, STK11, GNAS were mutated in 68 (65%), 37 (36%), 31 (35%), 2 (2%), 2 (2%), 1 (1%), 1 (1%), 1 (1%), 1 (1%) of the total 104 HCCs, respectively, and 92 (88%) of 104 HCCs had mutations either in TERT promoter, CTNNB1 or TP53. HBV integration was found in 93% (25/27) of HBsAg positive HCC by deep sequencing. TERT promoter mutations were associated with older age ($p = 0.017$), presence of hepatitis C virus infection ($p = 0.0026$) and absence of hepatitis B virus infection ($p < 0.0001$). TP53 mutations were found in hepatitis B virus infection ($p = 0.0002$) and absence of hepatitis C virus infection ($p = 0.0014$). CTNNB1 mutations were associated with absence of hepatitis B virus infection ($p = 0.010$). Moreover, TERT promoter mutation was significantly associated with shorter DFS ($p = 0.006$) and poor overall survival ($p = 0.048$).

Conclusions: Gene alterations in TERT promoter, CTNNB1, and TP53 are closely associated with HCC development, and mutation in TERT promoter is related to poor prognosis. These results are useful for diagnosis and predicting outcomes for patients with HCC.

P0333

AN EPIDEMIOLOGICAL ASSOCIATION BETWEEN THE TRENDS OF NUCLEOSIDE ANALOGUE PRESCRIPTION AND LIVER CANCER INCIDENCE FROM 1999 TO 2012 IN HONG KONG, CHINA

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Background and Aims: More than 85% of hepatocellular carcinoma (HCC) in Hong Kong, China is associated with chronic hepatitis B (CHB) infection. Nucleoside analogue therapy for CHB was introduced to Hong Kong since 1999. We determined whether there was a positive effect on the population incidence of liver cancer by the introduction of nucleoside analogue therapy up to 2012.
Methods: We obtained nucleoside analogue (lamivudine, adefovir, telbivudine, entecavir and tenofovir) prescription data (1999–2012) from the electronic health record system of the Hospital Authority, Hong Kong, which provides >90% of secondary and tertiary care to the Hong Kong population. We excluded prescriptions with concomitant anti-HIV medications. Liver cancer data from 1990 to 2012 was obtained from the population-based Hong Kong Cancer Registry. Using Poisson piecewise regression analysis, we compared rate trends of liver cancer incidence from the period of 1990 to 1998 with the period of 1999 to 2012.
Results: Nucleoside analogue prescription patient headcount increased from 329 prescriptions in 1999 to 5,942 prescriptions in 2006, then to 26,411 prescriptions in 2012. The increase rate of 1999–2006 and 2006–2012 were 802 and 3,411 prescriptions per year respectively ($p < 0.001$). Age-standardized incidence of liver cancer, using World Health Organization standard population 2000 as reference, dropped from 22.4 per 100,000 persons in 1998 to 15.4 per 100,000 persons in 2012. After adjusting for the change in local CHB prevalence over the study period, there was a decline in the age-adjusted liver cancer incidence for all patients (2.7%, $p < 0.001$, 95% CI 1.4–4.0%), male patients (3.5%, $p = 0.009$, 95% CI 1.1–5.9%) and female patients (2.0%, $p < 0.001$, 95% CI 1.1–2.9%). When analyzing specific age groups, the decline in liver cancer incidence was most significant among patients aged 50–59 years (male: 15.8%, $p = 0.006$, 95% CI 5.7–25.9%, female: 7.1%, $p = 0.001$, 95% 3.4–10.9%), and patients aged 30–39 years (male: 3.8%, $p = 0.008$, 95% CI 1.3–6.4%; female: 1.3%, $p = 0.004$, 95% CI 1.1–2.1%).
Conclusions: From this interim analysis, we demonstrated an ecological association of nucleoside analogue prescription with the incidence of liver cancer in a CHB-prevalent region, providing epidemiological evidence that nucleoside analogue therapy for CHB could reduce the risk of HCC. Further analysis will be performed for liver cancer mortality and for more a detailed comparison of different time periods.

P0334

COMPUTER-TOMOGRAPHY (CT) WITH MAPPING OF THE ARTERIAL ENHANCEMENT FRACTION (AEF) FOR SCREENING OF HEPATOCELLULAR CARCINOMA (HCC) IN PATIENTS WITH END-STAGE LIVER CIRRHOSIS

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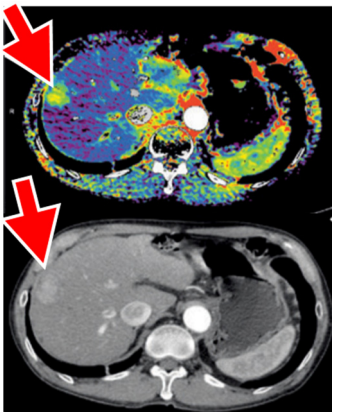
Background and Aims: CT imaging with arterial enhancement fraction (AEF) uses a tri-phasic CT image acquisition (unenhanced, arterial, portal venous phase) to generate color coded CT-perfusion images. The purpose of this study was to investigate if the use of the AEF imaging would supersede the acquisition of a fourth contrast phase (equilibrium phase) and thus allow a 25% reduction of radiation dose.
Methods: 55 patients who underwent liver transplantation between 2010 and 2013 and who had a CT scan acquired in four contrast phases (unenhanced, arterial, portal venous and equilibrium phase) on a Siemens Somatom Sensation 64 were included: 35 patients with 108 histologically proven HCC lesions, as well as 20 patients without HCC lesions. 47 lesions were already treated by prior TACE. AEF was calculated using the syngo.via workstation (Siemens, Erlangen, Germany): AEF = $[(HU_A - HU_U)/(HU_P - HU_U)] \times 100$, where HU is the attenuation, A the arterial phase, P the portal phase, and U the unenhanced scan. A total of 6 radiologists read the tri-phasic grayscale images in conjunction with the color AEF-maps. For the second read-out, three readers looked at the CT images with four contrast phases and three readers only at three contrast phases (without equilibrium phase).
Results: The JAFROC analysis showed a significant difference of the figures of merit (θ) between the tri-phasic read-out without ($\theta = 0.718$) and with the AEF-maps ($\theta = 0.750$, $p = 0.0007$). Diagnostic performance with four-phases ($\theta = 0.775$) was significantly better than with tri-phases ($\theta = 0.718$) without using the AEF-map ($p = 0.0018$), but not different from the tri-phasic CT with the AEF-maps ($\theta = 0.750$, $p = 0.3053$). Sensitivity of tri-phasic CT was 36.4% (95% CI: 32.5–40.5%) and PPV was 76.5% (95% CI: 71.0–81.4%). With the AEF-maps sensitivity and PPV increased to 49.7% (95% CI: 45.6–53.9%) and 83.4% (95% CI: 79.0–87.1%), respectively.
Lesions with and without prior TACE treatment were detected with a sensitivity of 31.9% and 42.3% without the use of the AEF map and with a sensitivity of 48.4% and 51.6% with the AEF map. A normalized AEF cut-off value of 70% for small lesions (<1 cm) and 60% for larger lesions (≥1 cm) leads to highest HCC-accuracy.
Conclusions: In HCC CT-screening the fourth equilibrium phase could be replaced by the calculated AEF-maps. This allows a 25% reduction of radiation dose, which is relevant for patient undergoing repetitive HCC-screening.



P0335

SERUM LONG CHAIN CERAMIDES, SPHINGOSINE AND SPHINGOSINE-1-PHOSPHATE AS NOVEL DIAGNOSTIC MARKERS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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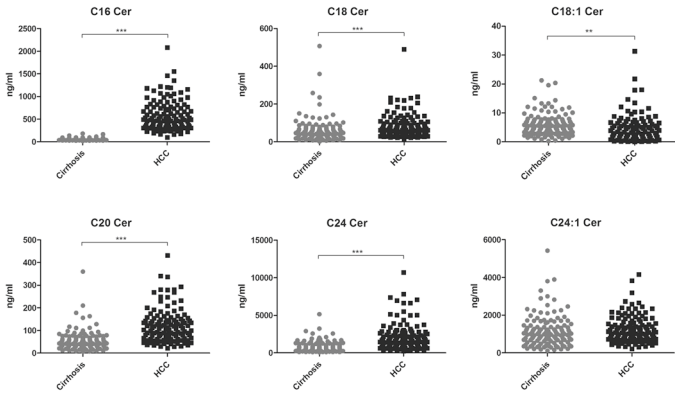
Background and Aims: We have recently shown that major alterations of serum sphingolipid metabolites in patients with chronic liver disease associate significantly with the stage of liver fibrosis in corresponding patients. Aim of the current study is to assess the serum sphingolipidomic profile of patients with HCC as

compared to liver cirrhosis und thus identify novel diagnostic tools potentially applicable in the clinical setting.
Methods: We assessed via mass spectrometry serum concentrations of sphingolipid metabolites in a series of 122 patients with HCC compared to an age- and sex-matched series of 127 patients with liver cirrhosis.
Results: We observed a highly significant upregulation of long and very long chain ceramides (C16-C24) in patients with HCC as compared to patients with liver cirrhosis ($p < 0.001$). Accordingly, dihydro-ceramides, synthetic precursors of ceramides and notably sphingosine, sphingosine-1-phosphate (S1P) and dihydro-S1P were upregulated in HCC as well as compared to liver cirrhosis ($p < 0.001$). Especially the diagnostic accuracy of C16-ceramide and S1P, assessed by receiver operating curve (ROC) analysis, showed a higher area under the curve (AUC) value as compared to α-fetoprotein (AFP), which constitutes the only available serologic biomarker of HCC so far (0.999 and 0.985 versus 0.823, $p < 0.001$ respectively).
Conclusions: Serum levels of long chain ceramides, sphingosine and S1P show a significant upregulation in patients with HCC as compared to patients with liver cirrhosis. Particularly C16ceramide and S1P may serve as novel diagnostic tools for the early identification of HCC in patients with liver cirrhosis.



P0336
IMPACT OF GENETIC VARIANTS OF TUMOR NECROSIS FACTOR LOCUS ON HEPATOCELLULAR CARCINOMA SURVIVAL
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Background and Aims: Genetic epidemiology indicates that cytokine polymorphisms related to cancer prognosis. This study aimed to assess the effect of polymorphisms of tumor necrosis factor (*TNF*) locus on survival in hepatocellular carcinoma (HCC).
Methods: Two hundred consecutive newly diagnostic cirrhotic patients with HCC were enrolled. Polymorphisms of *TNFα* −308, and *lymphotoxin (LT)* α +252 were genotyped by the polymerase chain reaction with direct sequencing or restriction fragmented length polymorphism. Kaplain-Meier method with log-rank test was used for univariate analysis of survival analysis. Cox proportional hazard regression meodel was used for multivariate analysis to assess independent factors related to survival.
Results: The frequency distribution of the genotypes of *TNFα* −308 and *LTα* +252 were [G/G (73.5%), G/A (26.5%) and A/A (0%)] and [A/A (34.5%), A/G (36.5%) and G/G (29.0%)], respectively. The variant genotypes (*TNFα* G/A and/or *LTα* G/G) were biomarkers for poor cumulative survival in patients with HCC (Kaplan–Meier method with log-rank test, $P = 0.0001$). The cumulative survival in patients with both variant genotypes (median, 0.62 year; 95% CI, 0.22–1.03 years; n = 33) was shorter than that in those without (median, 3.81 year; 95% CI, 3.09–4.52 year; n = 123, $P = 0.0001$) or those with either variant genotype [*TNF* 308 G/A, median, 2.48 (95% CI, 1.61–3.36) years, n = 19, $P = 0.01$; *LTα* G/G, median, 1.63 (95% CI, 1.48–1.71) years, n = 25, $P = 0.01$]. However, there was no significant difference in the cumulative survivals between patients with either variant genotype. Multivariate analysis with the Cox proportional hazard model indicated that the variant *TNFα* −308 G/A genotype [hazard ratio (HR), 2.55; 95% CI, 1.65–3.9 years] and *LTα* +252 G/G genotype (HR, 2.40; 95% CI, 1.54–3.74 years) were independent factors for poor HCC survival.
Conclusions: The variant genotypes *TNFa* −308 G/A and *LTα* +252 G/G were biomarkers for poor HCC survival.

P0337
A POLYMORPHISM IN IFNL3 IS AN INDEPENDENT RISK FACTOR FOR DEVELOPMENT OF HEPATOCELLULAR CARCINOMA AFTER TREATMENT OF HCV INFECTION
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Background and Aims: Polymorphism of the *IL28B* gene may predict the therapeutic response and outcome of chronic hepatitis C virus (HCV) infection. However, the impact of *IL28B* polymorphism on the development of hepatocellular carcinoma (HCC) after antiviral treatment remains controversial.
Methods: We retrospectively analyzed tissues from 1118 histologically proven HCV patients after peg-IFN/RBV therapies from 2000 to 2009 for *IL28B* polymorphism (rs12979860).
Results: The frequency of the *IL28B* rs12979860 CC, CT, and TT genotypes in chronic HCV patients was 86.4%, 13.2%, and 0.3%, respectively. The duration of follow-up ranged from 12–159 months, with a median of 60 months. At the end of follow-up, 108/1118 (9.66%) patients developed HCC. The *IL28B* CT/TT genotype was positively correlated with high baseline AFP levels (≥20 ng/mL), advanced fibrosis stage, diabetes (DM), and failure to attain sustained virologic response (SVR; all $P < 0.05$). Multivariate Cox regression analysis showed that age ≥60 y, low platelet count ($<15 \times 10^9$ cells/L), AFP ≥20 ng/mL, advanced fibrosis stage, DM, non-SVR and the *IL28B* CT/TT genotype were significant risk factors for HCC development ($P < 0.05$). Subset analysis revealed that age, platelet count, AFP levels, and fibrosis stage were risk factors for patients with SVR. The impact of *IL28B* was not significant although HCC occurred in SVR patients with the CT/TT genotype. In contrast, for patients without SVR, only fibrosis stage and the *IL28B* CT/TT genotype (HR: 1.80, 95% CI: 1.06–3.07, $P = 0.030$) were independent risk factors for HCC development.
Conclusions: The CT/TT *IL28B* polymorphism was associated with HCV-related HCC development, especially for patients without SVR after antiviral therapy.

P0338
SURVIVAL POST SORAFENIB IN HEPATOCELLULAR CARCINOMA: ARE AFP NON-SECRETORS A DISTINCT SUB-GROUP?
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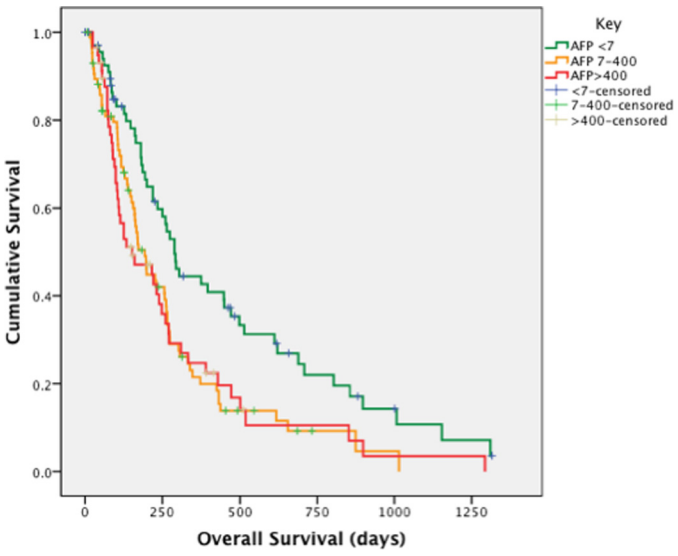
Background and Aims: Alfa fetoprotein (AFP) is the most commonly used marker for hepatocellular cancer (HCC), the serum concentration of which is elevated in about 70% of patients. An increase in AFP concentration up to 400 ng/ml has been reported in 10–15% of cases of acute and chronic hepatitis and liver cirrhosis, thus a level above 400 ng/ml in a high risk patient is generally accepted as diagnostic of HCC. Studies have shown that the higher

the AFP, the poorer the prognosis, but no good evidence in the literature exists on the minority of patients with HCC who do not secrete AFP. Our aim was to analyse this sub-group of patients from a cohort with advanced HCC treated with Sorafenib, and assess survival outcomes.

Methods: Consecutive patients with a confirmed diagnosis of HCC as per AASLD criteria, treated with Sorafenib at Queen Elizabeth Hospital Birmingham UK, between April 2009 and March 2014 were included in the analysis. We took our local reference range of <7 ng/ml as defining AFP non-secretory HCC. Electronic patient records were reviewed and variables including demographics, laboratory results, radiological data and survival outcomes were collected. Cox regression analysis was used to assess hazard ratios, and Kaplan–Meier methods were used for survival analysis.

Results: 217 patients were included in the analysis, consisting of 173 (79.7%) males and 44 (20.2%) females. The median age was 66 years (range 17–88) and 74 (34.1%) patients had received either RFA or TACE prior to Sorafenib. Median overall survival (OS) post Sorafenib was 7.2m (95% CI: 5.5–8.8m) and median progression free survival (PFS) was 4.8m (95% CI: 3.8–5.8m). 69 patients (32%) had AFP non-secretory HCC in this cohort. The median OS for the non-secretors was 9.5m (95% CI: 8.0–11.0) and for the secretor group it was 5.5m (95% CI: 4.0–7.1). The hazard ratio for survival when comparing these two groups was 1.78 (p = 0.0014). When analyzing survival in the secretor subgroup group with AFP between 7–400 ng/ml, little difference was noted between them and the group with APF >400 ng/ml (HR: 1.02, p = 0.89). The Kaplan–Meier curve presented shows the separation of survival curves for the 3 groups.

Conclusions: There is very little published in the literature about the AFP non-secretor HCC sub-group. Our data shows that this sub-group has better prognosis compared to patients with AFP secretory HCC. We believe that this data is important for clinicians and patients for prognostication and decision making.



P0340

CLINICAL PATTERNS OF HEPATOCELLULAR CARCINOMA (HCC) IN NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD): A MULTICENTER CASE–CONTROL STUDY

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) represents the hepatic manifestation of metabolic syndrome; its incidence is growing rapidly and can progress to hepatocellular carcinoma (HCC). Only scant clinical information on HCC in NAFLD is available.

The aim of the study was to assess the clinical features of patients with NAFLD-related HCC (NAFLD-HCC), and to compare them to those having HCV-related HCC.

Methods: The study is a multicenter observational case-control study. 756 patients with either NAFLD (145) or HCV-related chronic liver disease (611) have been enrolled in Secondary Care Italian Centers. Survival was modeled according to clinical parameters, lead time bias and propensity analysis.

Results: As compared to HCV, HCC in patients with NAFLD were less often male (61% vs 79%), had a higher BMI (29.1 vs 27.6) and metabolic syndrom components, had similar Child–Pugh score (5.6 vs 5.8), had a larger tumor volume (diameter largest nodule 4.1 vs 3.3 cm), more often showed an infiltrative pattern (15.4% vs 4.0%) and detection outside specific surveillance (52.4% vs 36.7%). Survival was significantly shorter (p = 0.017) in patients with NAFLD-HCC: namely 25.5 months (95% CI 21.9–29.1) in the NAFLD patients and 33.7 months (95% CI 31.9–35.4) in the HCV patients. This difference remained significant even after adjustment for lead time bias in surveilled patients. Cirrhosis was present in only about 50% of NAFLD-HCC patients. To understand the intrinsic aggressiveness of HCC and to eliminate possible confounders, a propensity score analysis was carried out considering age, gender, Child–Pugh score, largest tumor size, leading to 64 patients in each group. In this case, the difference in mean survival between the two groups (n= was no longer statistically significant, being 30.2 months (95% CI 25.3–35.2) in patients with NAFLD-HCC and 36.9 (95% CI 32.6–41.1) in patients with HCV-related HCC liver-related disease (p = 0.330).

Conclusions: NAFLD-HCC rises in the absence of cirrhosis in about 50% of the cases, and it is detected at a later tumor stage than in HCV-infected patients. Future research should identify patients with NAFLD who require surveillance in order to offer them the most timely and effective treatment.

P0341

DECREASING MORTALITY BY LIVER DISEASES BUT INCREASING INCIDENCE OF AND MORTALITY BY HEPATOCELLULAR CARCINOMA IN A HEPATITIS B VIRUS ENDEMIC AREA: NATIONWIDE WHOLE POPULATION DATA ANALYSES

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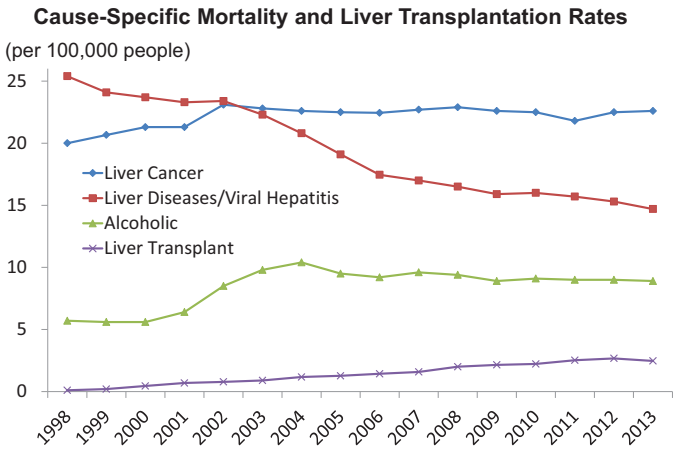
Background and Aims: The annual mortality rate by chronic liver diseases and hepatocellular carcinoma (HCC) that are associated with hepatitis B and C has been shown to be reduced by antiviral treatments. However, the prolonged life-expectancy of patients

with chronic viral hepatitis may increase the cumulative incidence and mortality rates by HCC.
Methods: Data on incidence rates of HCC were obtained from the Korea Central Cancer Registry, which compiles nationwide data on all newly diagnosed cancers. Data on cause-specific mortality were obtained from the Korean national death registry (Statistics Korea).
Results: The mean age at death by liver diseases and HCC significantly increased between 1998 and 2013 (56.3 years vs 64.8 years; $\beta = 0.65$, $p < 0.001$ and 60.0 years vs 63.9 years; $\beta = 0.30$, $p < 0.001$, respectively). Between 1998 and 2013, the mortality rate by liver diseases and viral hepatitis significantly decreased (25.4/100,000 people vs 14.7/100,000 people; $\beta = -0.77$, $p < 0.001$). The incidence rate of and mortality rate by HCC significantly increased during the same period (28.2/100,000 people vs 32.9/100,000 people; $\beta = 0.45$, $p < 0.001$ and 20.0/100,000 people vs 22.6/100,000 people; $\beta = 0.11$, $p = 0.01$). The trends in mortality rates by liver diseases and HCC were consistently observed after adjusting for alcoholic disease and liver transplantation.
Conclusions: During recent 15 years when the antiviral treatments for hepatitis B and C have been introduced, life-expectancy of patients with chronic liver disease has increased in a hepatitis B virus endemic area, which may be associated with the increasing nationwide incidence and mortality rates by HCC. There results suggest that HCC is becoming the main cause of death in patients with chronic viral hepatitis.



P0342
CANCER STEM CELLS AND VASCULOGENIC MIMICRY IN HEPATITIS C VIRUS-RELATED HEPATOCELLULAR CARCINOMA: RELATION TO TUMOR PROGRESSION

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Background and Aims: Accumulating evidence suggests that cancer stem cells (CSCs) play an important role in tumorigenicity and may be involved in tumor vascularization by forming non-endothelial vascular channels described as vasculogenic mimicry (VM). The aim of the present work was to study the expression of Epithelial cell adhesion molecule (EpCAM), a marker for CSCs and VM in HCV-related HCC in relation to tumor progression.
Methods: Twenty-five patients with HCV-related cirrhosis and HCC were enrolled in the study. The severity of liver disease was assessed according to Child–Pugh classification and the Model for End Stage Liver Disease (MELD) score. Tumor stage was classified according to the Barcelona Clinic Liver Cancer (BCLC) staging system and the Cancer of the Liver Italian Program (CLIP). Histological tumor grading was performed according to the Edmondson and Steiner and the adjacent non-neoplastic liver tissue was examined to assess METAVIR histological activity grade and stage. Immunohistochemical analysis was done in HCCs and adjacent non-neoplastic liver tissues using anti-human antibodies against EpCAM, alpha fetoprotein (AFP), hepatocyte for identification of VM and CD105 to quantify microvessel density (CD105-MVD).
Results: Positive immunostaining for EpCAM (>5% of cells) was demonstrated in 11 (44.0%) HCV-related HCCs while EpCAM expression in the adjacent non-neoplastic liver tissues was observed mainly in proliferating bile ductules in the portal tracts and/or in periportal hepatocytes. Vasculogenic mimicry was identified in 15 (60.0%) of HCC samples but in none of the adjacent non-neoplastic liver tissues. The cells lining the VM channels showed positive immunostaining for EpCAM. Compared with $EpCAM^-$ HCC patients, $EpCAM^+$ HCC patients showed significantly higher Child–Pugh class ($P = 0.036$), tumor size ($P = 0.006$), CLIP stage ($P = 0.004$) and histological grade ($P = 0.001$), AFP expression ($P = 0.024$) and VM formation ($P = 0.006$). Positive correlations were found between VM formation and tumor size ($P = 0.018$), CLIP stage ($P = 0.020$) and histological grade ($P = 0.025$). No significant correlations were found between EPCAM expression or VM formation with CD105-MVD.
Conclusions: EpCAM positivity may identify a subpopulation of HCCs originated from CSCs with aggressive biological behavior and may be involved in tumor vascularization through VM formation. Targeting CSCs as well as VM may be pivotal therapeutic options in the treatment of HCC.

P0343
THE PATTERN OF ALPHA-FETOPROTEIN LEVEL HAS GREATER DIAGNOSTIC VALUE COMPARED TO ISOLATED VALUES IN PREDICTING THE PRESENCE OF HEPATOCELLULAR CARCINOMA

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Background and Aims: It has been suggested that a very high serum alpha-fetoprotein (AFP) level in patients with chronic liver disease is diagnostic of hepatocellular carcinoma (HCC), however there is little data exploring other causes of such elevated AFP levels. In addition, there is limited data exploring the pattern of AFP elevation and its diagnostic utility in HCC.
Aims:
1. To determine the causes of an elevated AFP in a hospital setting
2. To determine the predictive value of an AFP greater than 100 μg/L, 400 μg/L and 1000 μg/L for diagnosing hepatocellular carcinoma (HCC)
3. To evaluate the diagnostic utility of the pattern of AFP change

Methods: All AFP values of 20 μg/L or greater were determined from the Eastern Health Pathology database from the 1st of January 2008 to the 1st of January 2013. Pregnant females and children were excluded. Electronic patient records were searched to determine the cause of the elevated AFP and peak AFP. Patients with at least three AFP readings over at least a three month interval were further analysed graphically and divided into one of four patterns; increasing, decreasing, fluctuating or stable.
Results: 195 patients were identified as having at least one AFP value of at least 20 μg/L, of which 22 (11%) were excluded (21 due to pregnancy and 1 due to infancy). 61 (35%) of the raised levels were attributed to HCC with other causes including hepatitis C (24%), hepatitis B (23%), germ cell tumours (9%), gastrointestinal (GI) malignancy (3%) and metastatic malignancy of unknown primary (3%). There were 81, 44 and 13 patients with a peak AFP of greater than 100 μg/L, 400 μg/L and 1000 μg/L, respectively, and these gave positive predictive values of 0.48 [95% confidence interval (CI)

0.37–0.59], 0.64 (95% CI 0.48–0.77) and 0.62 (95% CI 0.32–0.85), respectively, for the diagnosis of HCC. Other causes of an AFP greater than 400 included germ cell tumours (16%), GI malignancy (7%), hepatitis B (5%) and hepatitis C (5%). 67 patients had at least 3 AFP readings. An increasing pattern of AFP yielded a sensitivity of 0.55 (95% CI 0.36–0.74), a specificity of 0.85 (95% CI 0.48–0.79) for HCC. Hepatitis C (19%) and germ cell tumours (10%) were the only other causes of an increasing pattern of AFP.

Conclusions: An AFP of greater than 400 μg/L suggests a malignant process but is not specific for hepatocellular carcinoma. An increasing pattern of AFP in the absence of a germ cell tumour or hepatitis C is highly suggestive of hepatocellular carcinoma and warrants further investigation.

P0344

STREAMLINING HEPATOCELLULAR CARCINOMA SURVEILLANCE TO IMPROVE ITS PERFORMANCE: THE HEPATOMA AND COMPENSATED CIRRHOTIC FOLLOW-UP (HACC-FOL) PILOT PROGRAMME

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Background and Aims: The incidence of hepatocellular carcinoma (HCC) is increasing and patient outcomes depend critically on early diagnosis through surveillance of at-risk patients. The effectiveness of surveillance is adversely affected by patient, physician and administrative factors. We aimed to study whether a streamlined, nurse-led model of HCC surveillance could be implemented to improve outcomes.

Methods: Patients undergoing standard of care (SOC) HCC surveillance (6-monthly US +/− AFP measurement and specialist outpatient follow-up of results) were identified. A new model of care (the HACC-FOL programme) was piloted between July 2013 and June 2014. This comprised a prospectively implemented nurse-led telephone clinic, with a reminder sent to patients prior to their US and follow-up telephone call within 5 working days of surveillance investigations to assess patient symptoms and relay results. Informed consent was obtained in all cases and patients were returned to SOC follow-up after 1 cycle of surveillance. A staff hepatologist was immediately consulted regarding new symptoms or abnormal results, and appropriate diagnostic imaging was expedited if a lesion was detected on surveillance US. Patients completed satisfaction surveys at the start and end of the programme. Results were compared with those of SOC surveillance in the same patients over a 2 year period immediately before implementation of the HACC-FOL pilot.

Results: 130 patients were enrolled in the HACC-FOL pilot programme. In the 2 year period prior to its implementation, there were 26 episodes of failure to attend (FTA) surveillance US or follow-up appointments compared with only 3 FTA under the HACC-FOL programme. Six new lesions were detected requiring contrast imaging. Features of the SOC model "annoyed" or "distressed" patients, such as having to return to clinic for results (23%), excessive waiting times to see the doctor in clinic (13%) and not knowing results for weeks (14%). Patient satisfaction with the HACC-FOL programme was very high with improvements in perceived reporting of results, access to treatment and overall satisfaction.

Conclusions: The pilot HACC-FOL programme represents a streamlined, low-cost, nurse-led telephone clinic that improves clinical productivity and efficiency, reduces costs associated with FTA, and specifically addresses patient factors that may contribute to non-compliance. This innovative model represents an important initiative in an era of increasing mortality due the rising incidence of HCC.

P0345

THE ROLE OF CYTOKINES AND SEROMARKERS IN PREDICTING HCC RECURRENCE AFTER RADIOFREQUENCY ABLATION – INTERIM REPORT

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Background and Aims: Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. High recurrence rate after curative therapy may result in poor survival. Seromarkers such as VEGF, IGF-1 and cytokines including IL-γ, IL-5, IL-6, IL12, IP-10, fractalkine were found to be correlated with the outcome of HCC after therapies. The aim of the study is to investigate the prognostic role of seromarkers and cytokines in patients with HCC after radiofrequency ablation (RFA) with complete ablation.

Methods: This prospective study enrolls patients with HCC received RFA with complete ablation in our department. Patients were followed up every 3 months post treatment. The baseline liver biochemistry, age, gender, cirrhosis, etiology of HCC, viral load, history of anti-viral therapy, and baseline tumor parameters, as well as serum IL-γ, IL-5, IL-6, IL12, IP-10, fractalkine, VEGF and IGF-1 level were analyzed. Serum samples were stored at −80°C and tested by enzyme-linked immunosorbent assay (ELISA) (Quantikine Human IGF-1 and VEGF ELISA Kits; R&D Systems, Minneapolis, MN). Statistic analysis is done by SPSS 19. Independent student T test will be applied for normal distributed continuous variable while Mann–Whitney U test for non-normal distributed continuous variable analysis. Chi-square test will be used for categorical variable. AUROC was done for the goodness of the predictors.

Results: A total of 62 patients were recruited with a mean age of 69.4 year and 58.1% were male. HBV was detected in 34% while HCV as 53.2% of 62 patients. The median tumor size was 2.5 cm. 75.8% of HCC were TMN stage I while the rest were stage II. Only 26 (41.9%) of the 62 patients completed follow-up over 180 days, and 9 (34.6%) of 26 patients with additional intrahepatic HCC recurrence within 1 year. Those with higher IL-6 level tend to have high risk of recurrence (P = 0.021). The AUROC of IL-6 was 0.77 (95% CI: 0.571–0.969, P = 0.03). Moreover, if tumor size ≥3 cm, higher VEGF (AUROC: 0.833, 95% CI 0.635–1, P = 0.021) was more predictive than IL-6 (AUROC: 0.728, 95% CI 0.479–0.073, P = 0.117).

Conclusions: IL-6 is a pretreatment predictor for HCC with 1-year recurrence post RFA regardless of size while VEGF is more predictive of 1-year recurrence in HCC ≥3 cm. Further validation is necessary for the relative small sample size of this pilot study.

P0346

MULTICENTER ANALYSIS OF SOLUBLE Axl REVEALS DIAGNOSTIC VALUE FOR VERY EARLY STAGE HEPATOCELLULAR CARCINOMA

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Background and Aims: If diagnosed at early stages, patients with hepatocellular carcinoma (HCC) can receive curative therapies, whereas therapeutic options at later stages are very limited. Here we addressed the potential of soluble Axl (sAxl) as a biomarker of early HCC.
Methods: Levels of sAxl were analyzed by enzyme-linked immunosorbent assay in 548 serum samples from centers in Europe and China.
Results: Serum concentrations of sAxl were significantly increased in HCC (18.575 ng/mL) as compared to healthy (13.388 ng/mL) or cirrhotic (12.169 ng/mL) controls. Receiver operating characteristic curve analysis of sAxl in very early stage HCC patients (BCLC 0) showed an area under the curve (AUC) of 0.848, with a sensitivity of 76.9% and a specificity of 69.2%. α-Fetoprotein (AFP)-negative HCC patients displayed an AUC of 0.803, with sensitivity and specificity of 73% and 70.8%. Combination of sAxl and AFP improved diagnostic accuracy to 0.936 in very early HCC patients and to 0.937 in all HCC. Differential diagnosis of very early HCC versus liver cirrhosis showed a combined performance for sAxl and AFP of 0.901 with a sensitivity of 88.5% and a specificity of 76.7%. Furthermore, sAxl levels failed to be elevated in primary ovarian, colorectal and breast carcinomas as well as in secondary hepatic malignancies derived from colon.
Conclusions: sAxl outperforms AFP in detecting very early HCC as compared to healthy or cirrhotic controls and shows high diagnostic accuracy for AFP-negative patients. sAxl is specific for HCC and suggested as a biomarker for routine clinical use.

P0347

CORRELATION OF VARIOUS METABOLIC PARAMETERS OF ^{18}F-FDG PET WITH HISTOLOGIC FEATURES AND RECURRENCE OF HCC

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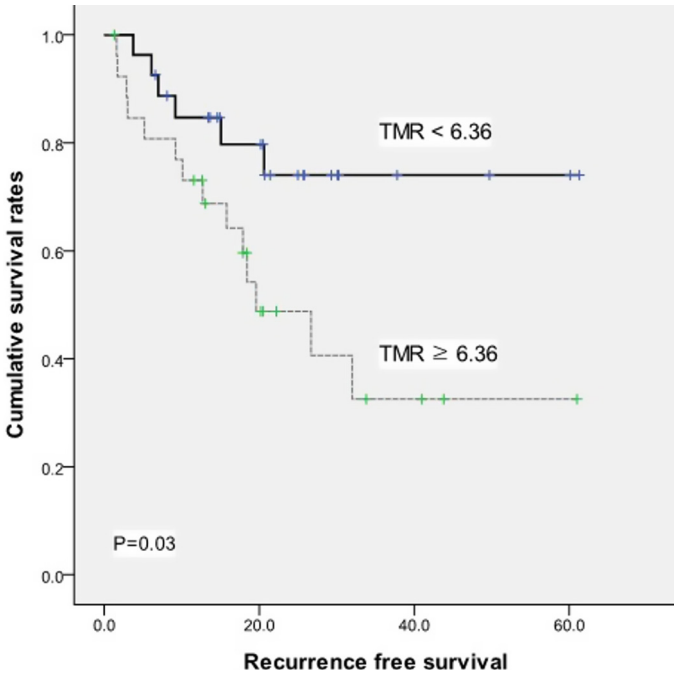
Background and Aims: Hepatocellular carcinoma (HCC) recurrence is observed in up to 70–80% of patients despite a curative treatment. Microvascular invasion (MVI) and poor differentiation are strong risk factors for recurrence, but these cannot be known preoperatively. The aim of this study was to investigate the correlation of ^{18}F-FDG PET with MVI and differentiation, and predictive role of tumor-to-background ratio of PET for recurrence in HCC.
Methods: Fifty-four patients including 45 males and 9 females had ^{18}F-FDG PET/CT study before surgical resection for HCC between December 2008 and December 2012. We analyzed the predictive role of clinicopathological features and metabolic parameters of ^{18}F-FDG PET/CT for recurrence of HCC. All 18F-FDG PET/CT scans were performed using a Discovery 710 PET/CT scanner (General Electric Medical System, Milwaukee, USA). Maximal standardized uptake value (SUVmax), tumor-to-nontumor ratio (TNR), tumor-to-muscle ratio (TMR) and tumor-to-blood ratio (TBR) were tested as metabolic index of ^{18}F-FDG PET.
Results: The mean follow-up duration was 31.48 months and postoperative recurrence developed in 20 (37%) patients. Twenty-seven patients (50%) had increased uptake in PET before surgical resection and 14 (51.9%) of them experienced the recurrence. Increased uptake in PET and TMR were associated with microvascular invasion (MVI) ($p = 0.04$, $p = 0.005$) and histologic differentiation ($p = 0.018$, $p = 0.002$). The median values of SUVmax, TNR, TMR and TBR were 3.22, 1.66, 6.36 and 2.24, respectively. Patients were classified into low and high groups using the mean values as cutoffs.
Patients were stratified and analyzed by univariate analysis using age, sex, tumor size, number, Child–Pugh classification, preoperative serum AFP, preoperative serum PIVKA-II, operative type, surgical resection margin, differentiation, capsule formation, septal formation, MVI, surgical resection margin, and SUVmax, TNR, TMR and TBR for metabolic parameters of PET. MVI was the only independent predictive factor for recurrence in multivariate analysis although TMR ≥6.36 showed a favorable result despite no statistical significance ($p = 0.061$). The cumulative recurrence-free survival (RFS) rates at 12 and 24 months were 79.7% and 74% in patients with TMR <6.36 and 64.2% and 32.5% in patients with TMR ≥6.36 ($p = 0.03$).
Conclusions: Increased 18F-FDG uptake of HCC, especially high TMR might be correlated with MVI and poor differentiation, and tends to have a risk for recurrence in HCC.



P0348

CAN HISTOLOGIC GRADE BE PREDICTED IN HEPATOCELLULAR CARCINOMA PATIENTS USING ENHANCEMENT DEGREE OF Gd-EOB-DTPA-ENHANCED MAGNETIC RESONANCE IMAGES? A PROSPECTIVE COHORT STUDY

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Background and Aims: Gd-EOB-DTPA (Primovist) is a liver-specific contrast agent, and Primovist-enhanced magnetic resonance images (MRI) has become clinically useful tool for diagnosis of small hepatocellular carcinoma (HCC). Several previous retrospective studies have tried to evaluate the association of histological grading with enhancement of HCC using MRI. In this study, we intended to evaluate whether histologic grade of HCC can be predicted using enhancement degree of Primovist-enhanced MRI in a prospective cohort with surgical resection for HCC.
Methods: A total of 71 patients who underwent surgical resection for HCC at our institution between January 2012 and March 2014 were prospectively enrolled. Primovist-enhanced MRI was performed in all patients before surgery. Signal intensities of HCC and peri-HCC areas were measured by using a region of interest. The relative intensity ratios of HCC lesion to the surrounding non-HCC area on unenhanced images (pre-contrast ratio) and those on hepatobiliary phase images (post-contrast ratio) were calculated.
Results: Of the 71 patients, Edmondson-Steiner grades I, II, III, and IV were observed in 5 (7%), 35 (49.3%), 28 (39.4%), and 3 (4.2%), respectively. Based on the Edmondson-Steiner grades I, II, III, and IV, mean pre-contrast ratios were 0.776, 0.773, 0.798, and 0.799, respectively, and mean post-contrast ratios were 0.673, 0.594, 0.599, and 0.597, respectively. These ratios showed no significant differentiation between each Edmondson-Steiner grade (each P-value >0.05).
Conclusions: Despite the liver-specific contrast agent of Gd-EOB-DTPA, enhancement degree of Primovist-enhanced MRI cannot provide useful information for prediction of histologic grade of HCC. Hasty decision on the differentiation of HCC based on the enhancement pattern of Primovist-enhanced MRI needs to be avoided.

P0349

sFRP-4, A POTENTIAL NOVEL SERUM MARKER FOR HEPATITIS B VIRUS-RELATED HEPATOCELLULAR CARCINOMA

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Background and Aims: To explore the diagnostic value of secreted frizzled related protein 4 (sFRP-4) in patients with hepatitis B virus (HBV)-related hepatocellular carcinoma.
Methods: Serum samples of 272 patients with chronic HBV infection [142 with hepatocellular carcinoma (HCC), 130 with chronic hepatitis B (CHB)] and 33 healthy controls were collected and divided into 2 sets. Meanwhile, 60 tissue specimens obtained from 30 HCC patients (30 specimens in foci, other 30 were precancerous tissues) were analyzed. Human antibody arrays were used for preliminary screening for serum markers in 15 patients (8 HCCs, 7 CHBs). Latent markers selected by human antibody arrays were assessed in 305 serum samples and 60 tissue specimens.
Results: Human antibody assays indicated that serum sFRP-4 levels were significant higher in HCC patients than in CHB patients ($P < 0.05$), and serum sFRP-4 levels were also significant higher in HCCs than in non-HCCs in test set [79.7 (54.3, 117.3) vs 41.3 (35.9, 56.2), $P < 0.001$] and validation set [89.0 (63.6, 131.1) vs 39.0 (28.9, 54.5), $P < 0.001$]. The area under the Receive Operating Characteristic curves (AUCs) were comparable between alpha-fetoprotein (AFP) and sFRP-4 [test set: 0.847, 95% confidence interval (CI): 0.788–0.906, vs 0.857, 95% CI: 0.796–0.918, $P > 0.05$; validation set: 0.868, 95% CI: 0.800–0.936, vs 0.0.889, 95% CI: 0.837–0.942, $P > 0.05$]. In the test set, sFRP-4 (sensitivity 94.4%, specificity 60.5% at 46.37 ng/ml) combined AFP (sensitivity 75%, specificity 84.9% at 11.3 ng/ml) resulted in a better performance with a sensitivity of 79.2% and specificity of 95.3%, and AUC of the two combination increased to 0.941 (95% CI: 0.908–0.975), similar results were seen in the validation group. Expression of sFRP-4 was higher in precancerous tissues than in focal lesions (22/30, 73.3%), there was no significant difference between precancerous tissues and focal lesions in the rest patients (8/30, 26.7%).
Conclusions: sFRP-4 is comparable to AFP for the diagnosis of HCC in CHB patients, their combination analysis can improve the diagnostic value.

P0350

KOREAN VERSION OF A MODEL TO ESTIMATE SURVIVAL IN AMBULATORY PATIENTS WITH HEPATOCELLULAR CARCINOMA (K-MESIAH)

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Background and Aims: A model to estimate survival in ambulatory hepatocellular carcinoma patients (MESIAH) is useful for estimating patient prognosis but needs improvement for Korean patients, most of whom have a hepatitis B virus. We aimed to modify the MESIAH for better prognostication through enhancing calibration for Korean patient population (K-MESIAH).
Methods: Utilizing a cohort of 1,969 hepatocellular carcinoma (HCC) patients from the National Cancer Center of Korea between 2004 and 2009, a survival prediction model was developed using the Cox proportional hazards model. The model's performance was evaluated using C-statistical and χ^2-statistical analyses. External validation was performed using an independent cohort of 328 patients from the Seoul National University Bundang Hospital.
Results: To develop the K-MESIAH, etiology was added to the original risk factors (age, Model for Endstage Liver Disease, albumin, size of the largest nodule, number of tumor nodules, vascular invasion, metastasis, and alpha fetoprotein) in the MESIAH. From the internal validation study, the C-statistics and χ^2-statistics for one-, three-, and five-years of survival were 0.83 (95% Confidence Interval: 0.82–0.85), 49.07; 0.81 (95% Confidence Interval: 0.79–0.82), 28.95; and 0.80 (95% Confidence Interval: 0.79–0.81), 20.93, respectively. The K-MESIAH also showed a high prediction ability for the external validation cohort.
Conclusions: A survival prediction model for Korean HCC patients was developed and validated to have a high level of performance. This K-MESIAH may be more useful in clinical practice and personalized care in a hepatitis B virus endemic area.

P0351

HIGHLY SENSITIVE α-FETOPROTEIN, *Lens culinaris* AGGLUTININ-REACTIVE FRACTION OF α-FETOPROTEIN AND DES-γ-CARBOXY PROTHROMBIN FOR HEPATOCELLULAR CARCINOMA DETECTION IN AN ITALIAN COHORT

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Background and Aims: Hepatocellular carcinoma (HCC) is a common complication in patients with chronic liver disease (CLD),

and particularly in those with cirrhosis. Currently, diagnosis and surveillance is mainly based on imaging-methods. However, recent technical improvements in the analytical methods of measuring α-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) and des-γ-carboxy prothrombin (DCP) employing an advanced microfluidics-based separation technology have been developed.

The aim of this study was to evaluate the diagnostic accuracy of α-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) and des-γ-carboxy prothrombin (DCP) alone and in combination, and to apply a recently proposed model (GALAD) that combines gender, age, AFP, AFP-L3 and DCP for the diagnosis of HCC in an Italian cohort.

Methods: A total of 98 patients [44 CLD patients without HCC (23M, 21F; mean age 53.2±13.4 years, 80% HBV-related) and 54 patients with HCC (45M, 9F; 69.5±9.8 years, 70% HCV-related)] were evaluated for AFP, AFP-L3 and DCP levels using highly sensitive assay on the μTASWako™ i30 auto-analyzer. Areas under the curve (AUC), sensitivity (Se), specificity (Sp) were calculated and compared to assess diagnostic performance of HCC markers and GALAD model.

Results: AFP, AFP-L3 and DCP values were significantly elevated in HCC compared to CLD patients [median AFP 13.4 (2.4–5785) ng/mL and 2.3 (1–25) ng/mL, p < 0.0001; median AFP-L3 5.9 (1–81.6) % and 1 (1–15.1) %, p < 0.0001; median DCP 1.13 (0.1–87.49) ng/mL and 0.265 (0.1–1.45) ng/mL, p < 0.0001; in HCC and CLD respectively]. AFP showed an AUC of 0.891 (cut-off 5.3 ng/mL, Se=0.81, Sp=0.86; 79.4% of patients correctly classified), AFP-L3 an AUC of 0.867 (cut-off 1%, Se=0.845, Sp=0.89; 84.5% of patients correctly classified) and DCP an AUC of 0.870 (cut-off 0.4 ng/mL, Se=0.78, Sp=0.91; 79.6% of patients correctly classified). The combination of the 3 markers resulted in AUC=0.947 (Se=0.94, Sp=0.86; 87.6% of patients correctly classified), whereas GALAD model showed AUC=0.976 (Se=0.96, Sp=0.84; 89.8% of patients correctly classified). Differences between AUC values and corresponding statistical significance are reported in Table 1.

Conclusions: Our data confirm the high analytical performance of the μTASWakoTM i30 tests for AFP, AFP-L3 and DCP quantitation. Moreover, the combination of these biomarkers and the use of GALAD model is superior to a single biomarker in HCC detection.

Table 1. AUC values comparison between AFP, AFP-L3, DCP, alone and in combination, and GALAD model

	DCP	AFP-L3	AFP + DCP + AFP-L3	GALAD model
AFP	ΔAUC = 0.024, p = 0.611	ΔAUC = 0.024, p = 0.551	ΔAUC = 0.056, p = 0.063	ΔAUC = 0.085, p = 0.011
DCP	/	ΔAUC = 0.003, p = 0.996	ΔAUC = 0.080, p = 0.002	ΔAUC = 0.106, p = 0.001
AFP-L3	/	/	ΔAUC = 0.080, p = 0.022	ΔAUC = 0.109, p = 0.002
AFP + DCP +AFP-L3	/	/	/	ΔAUC = 0.029, p = 0.028

Abbreviations: AFP, α-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; AUC, area under the curve; DCP, des-γ-carboxy prothrombin.

P0352
NEUTROPHIL LYMPHOCYTE RATIO (NLR) AT DIAGNOSIS IS A PREDICTOR FOR SURVIVAL IN PATIENTS RECEIVING SORAFENIB FOR ADVANCED HEPATOCELLULAR CARCINOMA (HCC): A LARGE UK COHORT

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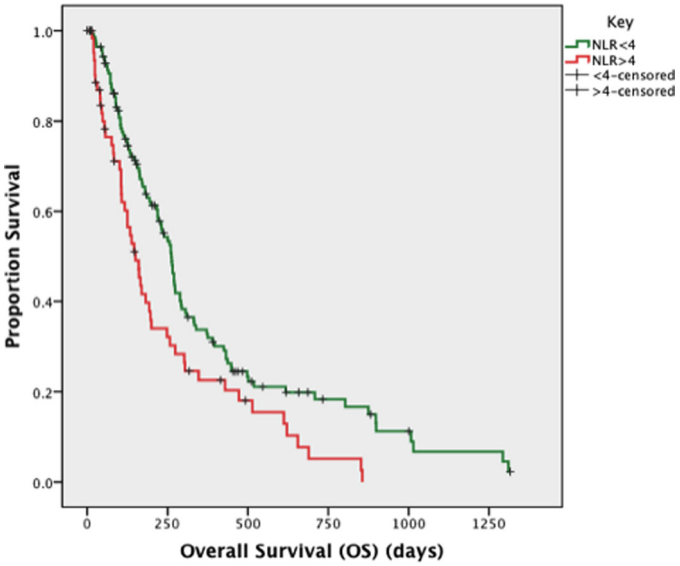
Background and Aims: Sorafenib is the current standard of care for patients with advanced or metastatic hepatocellular carcinoma. A body of evidence exists for the wide heterogeneity of response to sorafenib, but robust prognosticators in this setting are lacking. Neutrophil lymphocyte ratio (NLR) is a marker of systemic inflammation, and high NLR has been associated with poor response post TACE and RFA treatment, and with recurrence post transplantation. A small analysis on a Chinese population showed an NLR of >4 to be associated with poorer outcomes with sorafenib. Our aim was to perform a retrospective review of patients treated at a large academic cancer centre in the UK to assess the possible correlation between NLR and response to sorafenib.

Methods: All patients with a confirmed diagnosis of HCC, either by biopsy or radiologically as per AASLD criteria, who were treated with sorafenib at our centre between April 2009 and March 2014 were included in this analysis. Electronic patient records were reviewed and variables including demographics, hematological and biochemical laboratory results, radiological data and survival outcomes were collected. Cox regression analysis was used to assess hazard ratios, and Kaplan–Meier methods were used for survival analysis.

Results: 234 patients were included in the initial notes review. 17 were excluded at first review for unconfirmed diagnosis of HCC, or not having received sorafenib. The remaining 217 patients consisted of 173 (80%) males and 44 (20%) females. The median age was 66 years (range: 17–88) and 74 (34%) patients had received either RFA or TACE prior to sorafenib. Median overall survival (OS) post sorafenib was 7.2 mo (95% CI: 5.5–8.8 mo) and median progression free survival (PFS) was 4.8 mo (95% CI: 3.8–5.8 mo). Patients separated into 2 groups based on NLR of ≤4 or >4.0 derived diverging Kaplan–Meier curves for OS.

The median OS in those with NLR ≤4.0 was 8.6 mo (95% CI: 7.6–9.6 mo) and in those with NLR >4.0 it was 4.9 mo (95% CI: 3.5–6.3 mo). Corrected for AFP and the Child–Pugh score, patients with an NLR >4.0 had a 48% increased hazard of death than those with NLR ≤4.0 (HR 1.48, p = 0.041). The median PFS in those with NLR ≤4.0 was 5.5 mo (95% CI: 4.4–6.5 mo) and in those with NLR >4.0 was 3.4 mo (95% CI: 2.1–4.6 mo). The hazard ratio for progression between the two groups was 1.51 (p = 0.02).

Conclusions: NLR at diagnosis is a significant predictor for OS and PFS from initiation of sorafenib. We are currently undertaking validation of these findings utilizing an external dataset.



P0353
PROTEIN BIOMARKERS REACTIVE WITH HCC SERA SERVE AS A SIGNATURE FOR HEPATOCELLULAR CARCINOMA (HCC)

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Background and Aims: HCC is a known consequence of cirrhosis. Early recognition affords curative options, whereas delayed diagnosis has a poor prognosis. Despite current guidelines, many present with HCC at a non-curative stage. While biomarkers have been evaluated for screening and diagnosis, current biomarkers remain insensitive. A humoral response directed against oncoproteins or other aberrantly expressed proteins, has been documented in several malignancies. This immune response to neoantigen exposure might serve as a protein fingerprint for early and specific diagnosis.
Aim: Determine if an autoantibody signature exists to identify patients with HCC using patient's serum and established HCC cell lines as the reference
Methods: 40 patients with HCC (confirmed by histology or established radiologic features) were prospectively recruited from liver clinic. Sera were compared to 40 cancer-free patients with liver disease (LD) and 40 healthy controls (HC). Huh7 and HepG2 liver cancer cells were cultured, harvested and lysed. Proteins were extracted and separated by chromatofocusing. Fractions were further separated by non-porous silica according to hydrophobicity, collected in 96-well plates and deposited onto nitrocellulose slides generating protein microarrays. Arrays were hybridized with subject sera followed by fluorescently labeled goat anti-human IgG and scanned using a microarray scanner. Single-channel intensities of spots were determined. Replicated arrays were averaged and differences between cancer and normal serum were determined by non-parametric Wilcoxon rank-sum test p-value threshold 0.05. Quintile normalization was compared to averaged non-normalized data to detect consistent signals in HCC sera that were absent in LD sera and HC. Signal minimum of >1000 was accepted to eliminate low signal intensity. Prediction Analysis for Microarrays (PAM) was utilized for prediction analysis and validated with random forest.

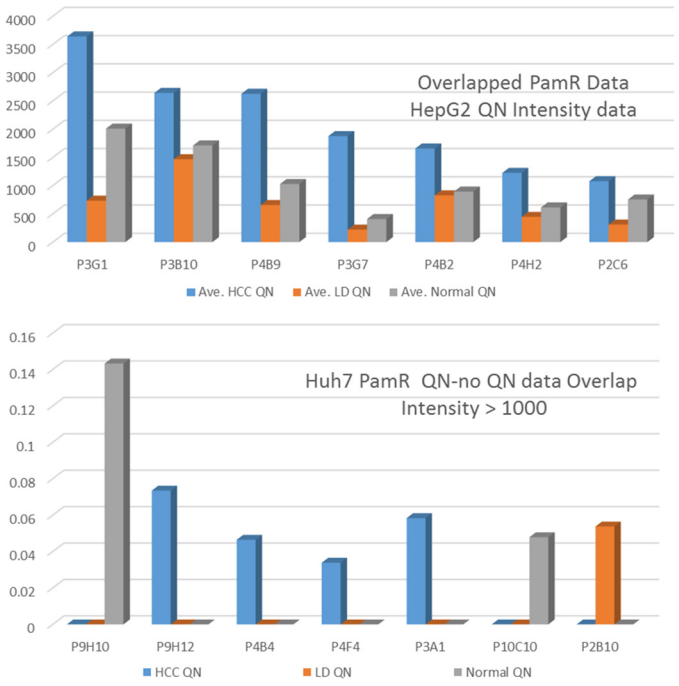

Figure 1.

Figure 2.

Results: Using the HepG2 cell line, 7 high-titer protein fractions were identified as potential markers to discriminate HCC from LD/HC (Figure 1). Similarly using Huh7 cell line, 2 high titer protein fractions were identified as potential markers (Figure 2). Mass spectrometry analysis is underway to identify potential reactive proteins.
Conclusions: Several candidate autoantibody biomarkers were identified using protein microarrays. These proteins will be further characterized and studied for their classification power using a larger sample set.

P0355
THE EVALUATION OF AN AUTOMATED RECALL PROGRAMME FOR SURVEILLANCE LIVER ULTRASOUND IN PATIENTS AT INCREASED RISK OF HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma is the fifth commonest cause of cancer death worldwide. The disease is often detected late when curative treatment is no longer possible. AASLD and EASL recommend six-monthly liver ultrasound in cirrhotic patients. The implementation of this is usually ad hoc from liver clinics, with risk of poor adherence.
Aims: To evaluate a radiology led, automated recall programme for HCC surveillance.
Methods: A single centre, retrospective cohort study of patients enrolled in the HCC surveillance programme at the Royal Liverpool Hospital from September 2009 to May 2013. All patients with cirrhosis Childs A-B, were included and hepatitis B patients with a family history of HCC. Patient demographics, cancer stage and cirrhosis etiology were recorded. Targeted liver ultrasounds were performed and rebooked for 6 months by administrative staff. The authors reviewed what happened to the cohort of patients.
Results: 6725 patients were identified. 804 patients were identified in the surveillance programme. Mean age 55 years (IQR 46–63 years), 497 male (62%). Aetiology: ALD 308 (38%), hepatitis C 154 (19%), hepatitis B 113 (13%), NASH 78 (10%), PBC 37 (5%), AIH 33 (4%), haemochromatosis 22 (3%), mixed 38 (5%). A total of

2366 scans were performed, for 100% adherence 3495 scans should have been done. 368 (46%) patients had complete surveillance, 182 (23%) did not attend 1 appointment. 81 (10%) patients had a USS suspicious for HCC. Of these 38 underwent CT, 56 had MRI, 2 contrast ultrasound and 14 both CT and MRI. 31 patients were diagnosed with HCC (3.9%), 19/31 (61%) were within Milan criteria, compared to the overall regional data 82/400 (21%), $p < 0.0001$, OR 0.49 (0.31–0.76). 9 patients had radiological curative disease, 9 were assessed for liver transplant. 4 patients were delisted for disease progression, two patients were not listed due to comorbidities, two were managed by ablation, and one patient was transplanted. 1 had a liver resection, 7 patients had ablations with curative intent. Of the other 10 patients, 6 were deemed too frail for any treatment, 3 had significant co-existent malignancies and 1 died of sepsis.

Conclusions: Patients in an automated USS surveillance programme had HCC detected at an earlier stage, but those receiving curative treatment were low. Adherence to surveillance was poor. A prospective randomized trial assessing the benefit, cost, and potential harm of surveillance is needed.

P0356

CIRCULATING FREE TUMOUR DNA CONCENTRATION IS A MORE USEFUL DIAGNOSTIC MARKER THAN TP53 MUTATION DETECTION IN HBV-RELATED HCC IN WEST AFRICAN PATIENTS

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Background and Aims: Hepatitis B and aflatoxin-related hepatocellular carcinoma (HCC) is common in West Africa, but access to HCC diagnostics such as CT and MRI are limited. This study evaluates circulating free tumour DNA (cfDNA) and aflatoxin-mediated TP53 249Ser gene mutation measurement in blood for HCC diagnosis.

Methods: 66 Nigerians had liver disease evaluation including liver ultrasound and blood tests. HCC was diagnosed by α-fetoprotein >400 IU/mL and characteristic findings on ultrasound or histology. CfDNA was isolated from 250 μL of plasma using QiaAmp circulating DNA kit (Qiagen). Plasma cfDNA was quantified with real time PCR using TaqMan TP53 249Ser Mutation detection assay (Invitrogen) and TP53 Reference assay, performed on a StepOnePlus instrument (Invitrogen).

Results: 26 patients had HCC (39%), 11 had HBV cirrhosis (17%), 11 chronic hepatitis B (17%) and 22 were healthy controls (33%). There were no significant clinical differences between groups on multivariate analysis. All HCC patients had stage IV disease. cfDNA concentrations were higher in HCC cases compared with controls (median cfDNA concentration 0.383 ng/μL, IQR 0.166–1.453 in HCC; 0.090 ng/μL, IQR IQR 0.044–0.239 in controls, $p = 0.0003$). ROC analysis of cfDNA concentration showed AUC = 0.766 for HCC diagnosis. Combining cfDNA concentration and α-fetoprotein levels (AFP) had AUC = 0.958 for HCC diagnosis. TP53 mutation was detected more frequently in HCC cases compared with controls (11 of 26 HCC compared with 6 of 48, $p = 0.006$) and mutated DNA concentration was higher in HCC cases compared with controls ($p = 0.004$). AUC for TP53 mutation concentration was lower than cfDNA concentration for diagnosis of HCC (AUC = 0.661). Furthermore, the addition of TP53 mutation concentration to cfDNA concentration did not appreciably improve AUC for HCC diagnosis (AUC = 0.772). cfDNA concentration was higher in patients with TP53 mutation ($p = 0.017$) and correlated with mutated DNA concentration ($p = 0.035$, $r = 0.52$). These factors were not independent diagnostic markers of HCC on logistic regression.

Conclusions: cfDNA levels are elevated in advanced HCC in West Africans and have moderate accuracy for HCC diagnosis. Though TP53 mutation is common in HCC, TP53 mutation detection appears dependent on cfDNA concentration and has lower utility for HCC diagnosis. Our data suggest cfDNA quantification alone or in combination with AFP may prove useful for HCC diagnosis in resource-poor settings.

P0357

AMINO ACID SIGNATURES IN RELATION TO THE RISK OF HEPATOCELLULAR CARCINOMA – A PROSPECTIVE CASE–CONTROL STUDY

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Background and Aims: Circulating levels of amino acid (AA) appear to be perturbed in hepatocellular carcinoma (HCC), but also in liver cirrhosis, obesity and diabetes, all of which are identified risk factors for HCC. However, from the current literature it is not clear whether observed associations are also apparent prior to HCC diagnosis. Therefore, we conducted a prospective study based on a large European cohort to characterise AA profiles in blood samples obtained on average 8.8 years prior to HCC diagnosis.

Methods: A nested-case control study was conducted within the prospective EPIC cohort of >520,000 participants from 10 European countries. During 8.8 years of follow up 147 HCC cases were identified and matched to 1 control each. AA levels in prospectively collected sera were measured with Biocrates AbsoluteIDQ-p180Kit. Log-transformed AA levels were z-standardized (Mean 0, SD 1). HBV/HCV status and liver enzyme levels were determined separately. Multivariable conditional logistic regression models were used to calculate odds ratios and 95% confidence intervals (OR; 95% CI) for individual AA, AA groupings and some of their specific ratios in relation to HCC risk.

Results: Six individual AA and two ratios were identified as the most predictive metabolites for HCC risk. The observed associations in multivariable models were inverse for leucine (OR = 0.46, 95% CI: 0.28–0.76), lysine (OR = 0.50, 95% CI: 0.32–0.78), glutamine (OR = 0.27, 95% CI: 0.13–0.56) and the ratio of branched chain to aromatic AA (OR = 0.27, 95% CI: 0.15–0.48; Fischer ratio). Phenylalanine (OR = 2.08, 95% CI: 1.32–3.26, $p = 0.01$), tyrosine (OR = 2.67, 95% CI: 1.65–4.30), glutamate (OR = 3.37, 95% CI: 1.82–6.24) and glutamate/glutamine ratio (OR = 4.32, 95% CI: 2.10–8.87) were positively associated with HCC risk. When the analyses were restricted to subjects diagnosed after 2 years from recruitment, all metabolites except phenylalanine maintained the associations. Controlling for hepatitis status or liver function markers had a confounding effect.

Conclusions: These findings based on sera collected up to many years prior to HCC diagnosis, show that circulating levels of aromatic (tyrosine, phenylalanine), branched chain (leucine), glutamate AA family (glutamine, glutamate) and lysine display strong HCC risk associations. The findings suggest that imbalances of specific AA and their impaired metabolism, possibly due to changes in liver function, may be involved in HCC development and may be further explored as potentially early predictors of HCC.

P0358

RISK PREDICTORS OF HEPATOCELLULAR CARCINOMA AMONG CIRRHOTIC PATIENTS CHRONICALLY INFECTED WITH HEPATITIS B

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Background and Aims: Various factors have been identified in determining the risk of hepatocellular carcinoma (HCC) and cirrhosis in patients chronically infected with the hepatitis B virus (HBV). However, risk factors for HCC in cirrhotic patients are still unclear. We examined HCC risk in cirrhosis patients based on genetic factors, as well as seromarkers measured closest to cirrhosis diagnosis during follow-up.
Methods: The REVEAL-HBV cohort enrolled 4,155 HBsAg seropositive people from Taiwan who were followed up every 6–12 months until 30 June, 2004. At entry and follow-up visits, blood samples were tested for serological markers. Covariates included in the model were age, HBeAg serostatus, qHBsAg levels, alanine aminotransferase (ALT) concentration, alpha-fetoprotein (AFP) level, and the *ALDH2* rs671 SNP variant. Cox proportional hazards models were used to estimate the risk of HCC associated with different factors.
Results: A total of 301 people developed cirrhosis during follow-up. Of those 301 people, 76 were diagnosed with HCC during 2,462 person-years of follow-up. Compared to those who were diagnosed with cirrhosis between ages 30 and 39, patients aged 60 and older had an adjusted hazard ratio (HR [95% CI]) of 14.26 (3.17–64.08) [p = 0.0005] of developing HCC. Compared to ALT levels <15 U/L, those with ALT levels ≥45 U/L exhibited an adjusted HR (95% CI) of 3.68 (1.70–7.99) [p = 0.0010]. With an AFP level ≥20 ng/mL, the adjusted HR (95% CI) was 3.52 (1.78–6.93) [p = 0.0003] compared to AFP <10 ng/mL. HBeAg(+) participants showed an adjusted HR (95% CI) of 2.85 (1.49–5.46) [p = 0.0015] when compared to HBeAg(−) individuals with qHBsAg levels <1,000 IU/mL. Those with any genetic variation (AA or AG) of the *ALDH2* rs671 SNP had a protective effect with an adjusted HR (95% CI) of 0.35 (0.20–0.59) [p < 0.0001], when compared to the GG genotype.
Conclusions: Older age, the GG *ALDH2* variant, HBeAg seropositivity, high HBsAg levels, high ALT levels, and high AFP levels near cirrhosis diagnosis were associated with an increased risk of progression from cirrhosis to HCC in chronic HBV infected participants.

P0359

ESM-1 IS A MARKER OF MICROVASCULAR INVASION, SATELLITE NODULES, AND ADVERSE CLINICAL OUTCOME IN PATIENTS WITH HEPATOCELLULAR CARCINOMA TREATED BY SURGICAL RESECTION

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Background and Aims: The prognosis of patients with hepatocellular carcinoma (HCC) eligible for curative treatment who undergo surgical resection or radiofrequency ablation (RFA) is highly impacted by the detection of satellite nodules and/or microvascular invasion. However, these features are identified on surgical samples and can neither be detected by liver imaging, nor by examination of liver biopsy. We previously demonstrated that immunohistochemical expression of Endothelial Cell-Specific Molecule 1 (ESM-1) by endothelial stromal cells predicted early recurrence of HCC after radiofrequency ablation (RFA). The aim of the present study was to assess the frequency and the distribution of ESM-1 expression in surgical samples of HCC eligible for curative treatment, to correlate its expression to microvascular invasion, satellite nodules and to clinical outcome.
Methods: One hundred and fifty-one HCC cases treated by surgical resection and classified BCLC 0 (n = 7, 5%), A (n = 108, 71%), or B (n = 36, 24%) from 2 University Hospital were included. Clinical, biological, pathological and follow-up data were systematically recorded. Expression of ESM-1 was investigated by immunohistochemistry. Survival and time to recurrence were analyzed using Kaplan–Meier method, log-rank test and Cox univariate and multivariate analysis.
Results: ESM-1 expression was observed in 48% (72/151) of the cases. It was strictly restricted to stromal endothelial cells and regularly distributed throughout the tumor sample. The detection of ESM-1 positive stromal cells was highly related to microvascular invasion (p = 0.002) and satellite nodules (p < 0.001). In univariate analysis, ESM-1 expression was a predictive factor of early recurrence (p = 0.0147) and overall survival (p = 0.0539). In multivariate analysis, the only factor associated to disease-specific survival was the existence of satellite nodule (p = 0.002).
Conclusions: ESM-1 expression by endothelial stromal cells, detected in 48% of BCLC 0/A/B HCC surgical samples is highly related to the presence of satellite nodules and microvascular invasion. These findings strongly suggest its use as a simple and reliable prognosis marker in biopsy samples.

P0360

STAT6 rs3024974 MIGHT PREDICT WORSE PROGNOSIS IN HEPATOCELLULAR CARCINOMA PATIENTS

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Background and Aims: The signal transducer and activator of transcription (STAT) 4 and 6 belong to STAT protein family. It has been demonstrated to be associated with autoimmune diseases. Recently, a genome-wide association study linked STAT4 rs7574865 with hepatocellular carcinoma (HCC) development. While another polymorphism of STAT4, rs3821236, which has comparable effect

with rs7574865, has seldom investigated. Besides, the role of STAT6 in HBV related diseases, including HCC, received scant attention.
Methods: In total, we recruited 948 Chinese Han subjects, including 220 HCC patients, 383 chronic HBV carriers and 345 natural clearance subjects. Four single nucleotide polymorphisms (SNPs) (STAT4 rs3821236, STAT6 rs324011, rs3024974, rs703817) were genotyped through high-resolution melting curve method.
Results: All the SNP distributions were in Hardy–Weinberg equilibrium (P > 0.05). In HCC patients, clinical characteristics including tumor size (5 centimetre as the cut-off), tumor number (single vs. multiple), portal vein tumor thrombus (Yes vs. No), distant metastasis (Yes vs. No) and portal vein invasion (Yes vs. No) were taken into consideration. STAT6 rs3024974 non-CC genotypes had a higher probability to develop tumor thrombus (OR = 2.75, 95% CI=1.30–5.84, P = 0.007) and portal vein invasion (OR = 2.34, 95% CI=1.08–5.07, P = 0.028). While no other polymorphisms (rs324011, rs703817 and rs3821236) associated with these HCC clinical characteristics. Additionally, we appraised whether these polymorphisms correlated with HBV susceptibility and HCC development. Results indicated that no polymorphisms of STAT4 and STAT6 were associated with HBV chronicity (P > 0.05) as well as HCC development. Then we combined HCC group with HBV group, and designated all the patients as the HBV carrier group. In comparison with natural clearance subjects, STAT4 rs3821236 GA genotype seemed to be a protective factor against HBV chronicity (OR = 0.61, 95% CI=0.45–0.83, P = 0.002), but allele model showed that allele A did not correlate with HBV chronicity (OR = 0.90, 95% CI=0.74–1.08, P = 0.267). No other SNPs or genotypes presented significant associations with HBV chronicity.
Conclusions: STAT6 rs3024974 associated with tumor thrombus and portal vein invasion and thus might predict, to some extent, worse outcome of HCC patients. However, all the selected polymorphisms were not explicitly associated with HBV susceptibility or HCC development and further high-quality studies were still necessitated.

P0361
HOST GENETIC POLYMORPHISMS WITH HEPATOCELLULAR CARCINOMA RISK IN CHINESE HAN POPULATION: A CASE–CONTROL STUDY

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Background and Aims: Human Leukocyte Antigen (HLA) DP/DQ polymorphisms have been demonstrated to be associated with hepatitis B virus (HBV) clearance in different races in our previous study. And Signal Transducer and Activator of Transcription 4 might associate with HBV natural clearance in Tibetans. Till now, no studies concurrently investigated the association between the two genes with the pathological variables of the cancer.
Methods: In total, we enrolled 629 subjects into this study, including 379 chronic HBV patients and 250 HCC patients. Four single nucleotide polymorphisms (SNPs) (HLA-DP rs3077, rs9277535, HLA-DQ rs7453920 and STAT4 rs7574865) were genotyped through high-resolution melting curve method. Comparison was made between HBV and HCC patients to validate whether these SNPs associate with clinical traits of HCC.
Results: The distribution of all the polymorphisms was in Hardy–Weinberg equilibrium. We stratified all the HCC patients based on clinical characteristics including tumor size, portal vein tumor thrombosis and distant metastasis. Results indicated that rs7453920 allele A associated with increased risk of portal vein tumor thrombosis (OR = 6.92, 95% CI=3.68–13.04, p < 0.001), though this allele has been regarded as the protective allele toward HBV persistent infection. And STAT4 rs7574865 allele T correlated with reduced risk of portal vein tumor thrombosis (OR = 0.15, 95% CI=0.06–0.37, p < 0.001) and tended to increase the risk of a large tumor size (OR = 1.51, 95% CI=0.99–2.28, p = 0.053) (See Table 1). But none of all the SNPs connected with tumor size, distant metastasis as well as the venous invasion (P > 0.05).
Conclusions: Currently selected SNPs of HLA-DP/DQ, STAT4 seemed not to correlate with HCC development based on our present study. But STAT4 rs7574865 and HLA-DQ rs7453920 might correlate with portal vein tumor thrombosis and thus could predict the prognosis of HCC patients.

P0362
HEPATOCELLULAR CARCINOMA INCIDENCE IN CHRONIC HEPATITIS C PATIENTS ACCORDING TO ANTIVIRAL TREATMENT STATUS

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Background and Aims: Our aim was to assess the hepatocellular carcinoma (HCC) incidence during the time and HCC stage at the diagnosis in relation to antiviral treatment status.
Methods: We retrospectively collected data of HCV patients, who were diagnosed between 1989–2011 and treated at the AKH-University Hospital of Vienna. We analyzed the HCC incidence as well as the BCLC stage at the time of HCC diagnosis during the follow-up in HCV patients according to the HCV-treatment status.

Table 1 (abstract P0361). Correlation of HLA-DQ rs7453920 and STAT4 rs7574865 with clinicopathological characteristics in patients with HCC

Clinical characteristics	rs7453920				rs7574865			
	G (%)	A (%)	OR (95% CI)	p	G (%)	T (%)	OR (95% CI)	p
Tumor size								
≤5 cm	199 (94.8)	11 (5.2)	1.00		154 (73.3)	56 (26.7)	1.00	
>5cm	197 (91.2)	19 (8.8)	1.75 (0.81–3.76)	0.151	137 (64.6)	75 (35.4)	1.51 (0.99–2.28)	0.053
Tumor number								
single	286 (94.7)	16 (5.3)	1.00		205 (68.3)	95 (31.7)	1.00	
multiple	158 (91.9)	14 (8.1)	1.58 (0.75–3.33)	0.222	117 (68.8)	53 (31.2)	0.98 (0.65–1.47)	0.912
Portal vein tumor thrombosis								
No	289 (92.6)	23 (7.4)	1.00		211 (68.1)	99 (31.9)	1.00	
Yes	49 (64.5)	27 (35.5)	6.92 (3.68–13.04)	<0.001*	73 (93.6)	5 (6.4)	0.15 (0.06–0.37)	<0.001*
Distant metastasis								
No	401 (93.7)	27 (6.3)	1.00		295 (69.2)	131 (30.8)	1.00	
Yes	42 (95.5)	2 (4.5)	0.71 (0.16–3.08)	0.643	28 (66.7)	14 (33.3)	1.13 (0.57–2.21)	0.73
Venous invasion								
No	378 (93.1)	28 (6.9)	1.00		279 (69.4)	123 (30.6)	1.00	
Yes	68 (97.1)	2 (2.9)	0.40 (0.09–1.71)	0.199	46 (65.7)	24 (34.3)	1.18 (0.69–2.02)	0.539

*Values are regarded as statistically significant.

Results: We identified 2627 HCV patients in our Department. We had to exclude 126 patients due to insufficient follow-up data and 64 patients diagnosed with HCC simultaneously to the HCV-infection. Thus in the final analysis we included 2437 patients.
The overall HCC incidence after a median follow-up of 8 years (range: 0.5–24) was 10.3%, being significantly lower in patients who received antiviral treatment as compared with untreated patients: 5.9% vs. 19.1%, p < 0.0001. Also, the HCC incidence in the treated patients was significantly lower in patients who achieved a sustained virologic response (SVR) as compared with non-SVR patients: 1.8% vs. 12.6%, p < 0.0001.
In the group of SVR patients, overall the HCC incidence/year of follow-up was 0.2%; the annual HCC incidence according to the number of treatment until achieving SVR was: 1 treatment – 0.1%, 2 treatments – 0.5% and 3 treatments – 0.15% (p = 0.008).
The overall HCC incidence/year of follow-up in the non-SVR group was 1.25%; the annual HCC incidence according to the number of treatment received, was: 1 treatment – 1.3%, 2 treatments – 1.3%, 3 treatments – 0.9% and >3 treatments – 1.1% (p = 0.58).
The HCC incidence/year of follow-up in the untreated group was 11.6% (untreated vs. treated: p < 0.0001; comparison SVR vs. non-SVR: p = 0.01).
The median time between HCV and HCC diagnosis was 7 years (2–14) in SVR patients, 6 years (1–21) in non-SVR and 3 years (0.5–21) in non-treated patients.
The BCLC stage at the moment of HCC diagnosis in all patients, SVR, non-SVR and non-treated patients are presented in Table 1.

Table 1. BCLC stages at the time of diagnosis in different groups of patients in relation to the anti-HCV therapy status

BCLC stage	All patients (n = 2437)	SVR patients (n = 1011)	Non-SVR patients (n = 610)	Untreated patients (n = 816)
0	0.8%	0%	1.4%	0.6%
A	23.4%	31.5%	32.4%	17.8%
B	23.7%	26.3%	23.4%	23.6%
C	33.2%	36.9%	29.9%	34.4%
D	18.9%	5.3%	12.9%	23.6%

Conclusions: The patients, who received antiviral treatment, especially those with treated successful had a significantly lower HCC incidence and more cases of HCC were diagnosed in early stages, comparable with non-treated group.

P0363
SYSTEMATIC REVIEW AND META-ANALYSIS OF INCIDENCE AND RISK FACTORS OF HEPATOCELLULAR CARCINOMA IN UNTREATED HBV INFECTED PATIENTS

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Background and Aims: The risk of hepatocellular carcinoma (HCC) in chronic hepatitis B virus (HBV) infection may vary in different clinical settings and not completely defined. We aimed to assess incidence rates, and factors related with occurrence, of HCC in untreated subjects with HBV chronic infection.
Methods: We performed a systematic review and meta-analysis searching for published articles in Medline, Embase, and the Cochrane Library up to October 2014 as well as in reference literature. We included longitudinal studies assessing HCC incidence in untreated HBV infected patients. The HCC estimates incidence rates (IRs) and their 95% confidence intervals (95% CIs) were extracted by each study and pooled together in random effects models. Assessment of heterogeneity and meta-regression analyses were done.

Results: 79 studies were included with a total of 363,838 participants and 3,472 HCC cases. In asymptomatic carries, the IRs of HCC were 0.04 (95% CI: 0.012–0.07), 0.19 (0.07–0.31) and 0.44 (0.23–0.66) per 100 person-years in Europe, North America and East Asia, respectively. In inactive carriers and subjects with chronic hepatitis, IRs were 0.02 (0.0–0.06) and 0.12 (0.03–0.27) in Europe, and 0.05 (0.03–0.06) and 0.49 (0.32–0.66) in East Asia, respectively. In compensated cirrhosis (Child–Pugh A), IRs were 2.03 (1.30–2.77), 2.89 (1.23–4.55) and 3.62 (2.65–4.58) in Europe, North America and East Asia, respectively. Meta-regression showed a statistically significant increase of IRs for age, male percentage, in North America and East Asia with respect to Europe, and in asymptomatic carriers, and patients with chronic hepatitis and compensated cirrhosis with respect to inactive carriers (Figure). Few studies, almost all performed in East Asia, investigated the role of alcohol, tobacco, diabetes and obesity and HBV genotype, so as no precise evaluation of their roles in progression of HBV infection to HCC could be done.

Variable	IR increase per 100 p-y	95% CI	p-value
Age (10 years)	1.09	1.05–1.14	<0.001
Gender (% males)	1.02	1.00–1.03	0.02
Area			
Europe	Reference		
North America [a]	3.10	1.00–9.98	0.05
East Asia	2.24	1.04–4.82	0.04
Disease status			
Inactive carrier	Reference		
Asymptomatic carrier	1.84	0.92–3.66	0.08
Chronic hepatitis	7.84	3.44–17.98	<0.001
Compensated cirrhosis	18.92	9.93–35.71	<0.001

[a] 70% Asiatic subjects.

Conclusions: The risk of developing HCC in untreated subjects with HBV chronic infection is strongly related with age, gender, area of residence, and HBV disease status.

P0364
CLINIC EVALUATION OF CIRCULATING MICRORNAS AS POTENTIAL BIOMARKERS OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH HBV CHRONIC INFECTION

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Background and Aims: Several studies showed that aberrant miRNA expression is associated with development and progression of hepatocellular carcinoma (HCC). Because of their stability in the circulation, miRNAs have been proposed as potential biomarkers of HCC.
The aim of this study was to examine whether some commonly deregulated miRNAs (miR-122, miR-21, miR-221 and miR-16) in HBV-related HCC may serve as diagnostic markers.
Methods: Serum expression of miRNA miR-122, miR-21, miR-221 and miR-16 was evaluated by real-time quantitative RT-PCR in 90 subjects: 33 patients with HBV-related HCC (age 61±10; F/M = 4/29), 30 patients with HBV-related cirrhosis (age 53±11; F/M = 11/19) and 27 blood donors as healthy controls (age 54±8; F/M = 9/18). Relative expression was correlated with clinical features among patients and controls.
Results: Median levels of miR-16, miR-122 and miR-221 were significantly different in patients with HCC or cirrhosis than in healthy controls (p < 0.001) whereas, only miR-122 levels differed in patients with HCC from cirrhotic patients (p = 0.024). Expression levels of mir-21 were similar in the 3 groups. miR-122 levels were significantly higher in patients with multifocal HCC than in patients with a single lesion (p = 0.024).

Area under the curve (AUC) analyses showed that serum levels of miR-122, miR-122 + miR-221, miR-122 + miR-16 and miR-122 + miR-221 + miR-16, are able to differentiate patients with HCC from patients with cirrhosis (AUC = 0.675; AUC = 0.704; AUC = 0.681; AUC = 0.703, respectively). Moreover, miR-16, miR-122 and miR-221, alone or in combination, were potential markers for discriminating HCC patients from healthy controls (AUC >0.9) and for discriminating patients with cirrhosis from healthy controls (AUC >0.9). In addition, a positive correlation between miR-122 levels and HCC nodules number (R = 0.390; p = 0.036), and a correlation between miR-16 and miR-122 levels, and ALT values (R = −0.464, p = 0.034; R = 0.449, p = 0.536, respectively) was found.
Conclusions: Among the four microRNAs analyzed, miR-122 significantly discriminates patients with HCC from cirrhotic patients and patients with HCC or cirrhosis from controls. miR-122 appears to reflect liver necro-inflammation, since we observed a positive correlation with ALT levels. Moreover, our study showed a correlation between miR-122 expression levels and HCC multifocality, suggesting the possible use of this miRNA for tumor stadiation. Nevertheless, miR-122 AUC values were not sufficiently high for HCC screening purposes in clinical practice.

P0365
EFFECTS OF METFORMIN ON CLINICAL OUTCOME IN ADVANCED HCC PATIENTS RECEIVING SORAFENIB

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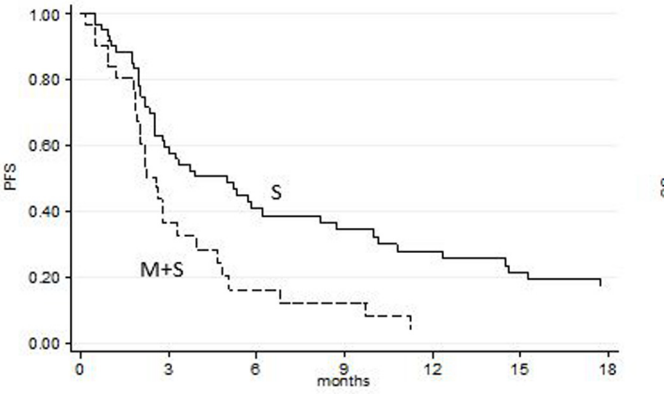
Background and Aims: Several studies reported an association between type 2 diabetes mellitus (DM2) and hepatocellular carcinoma (HCC). Data from several retrospective studies and meta-analysis have demonstrated a risk reduction of about 50% of developing HCC in cirrhotic patients treated with metformin for DM2. Several in vitro studies have shown anti-tumor activity of metformin (M) in HCC. The aim of this study is to evaluate the different outcomes of patients that have received or not metformin during treatment with sorafenib (S).
Methods: From 257 patients diagnosed with HCC from 2004 to 2014, 93 patients consecutively treated with sorafenib were analyzed. Of these, 42 patients (45.2%) were diabetic and 31 (33.3%) of these have received M for DM2. Progression-Free Survival (PFS), Overall Survival (OS) and their 95% Confidence Interval (95% CI) were estimated by Kaplan–Meier method and compared with log-rank test.
Results: The concomitant use of S and M was associated with a median PFS of 2.6 months (95% CI 1.9–3.3) compared to 5.0 months (95% CI 2.5–8.2) for patients who have not received M ($p = 0.029$) (See Fog 1). The concomitant use of S and M was associated with a median OS of 10.4 months (95% CI 3.9–14.4) compared to 15.1 months (95% CI 11.7–17.8) for patients who have not received M (p = 0.014).
Conclusions: These findings could be explained by an increased tumor aggressiveness and resistance to S in patients treated with M. M usually enhanced the activity of S, but probably molecular alterations in transporter genes or transcription factors involved in M molecular action and pharmacokinetics could contribute to a different response to these drugs combination. Further studies are needed to confirm the data and to identify possible mechanisms of resistance that may occur during treatment with S.



P0366
HEPATITIS C TREATMENT AND FIBROSIS STAGE IN SUBJECTS WITH HEPATOCELLULAR CARCINOMA AND HEPATITIS C IN A SINGLE U.S. CENTER VETERAN POPULATION

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Background and Aims: It has been shown that subjects with increased fibrosis stage and those who failed to eradicate hepatitis C (HCV) have higher rates of hepatocellular carcinoma (HCC). Our aim was to study treatment and fibrosis stage in subjects with HCC and HCV infection.
Methods: We identified 4537 unique subjects in the Minneapolis VAMC Hepatitis C Clinical Registry with HCV infection from years 1997 to October 31, 2014. We conducted a retrospective chart review of 208 subjects from that Registry with HCV infection and HCC: we assessed their demographics, history of HCV treatment and response, and fibrosis stage. We compared the proportion of subjects who developed HCC according to successful response to treatment, treatment failure, or lack of treatment.
Results: 208 of the subjects had HCC in the setting of HCV infection from the Minneapolis VAMC Hepatitis C Clinical Registry (n = 4537). The median age was 60 years (range 46 to 79 years). All 208 subjects were male. 12/208 (5.8%) developed HCC following treatment and achievement of a sustained virologic response (SVR); all of these were cirrhotic. 196/208 (94.2%) who developed HCC were never treated or had failed treatment; of these, 79/196 (40%) did not have cirrhosis.
Conclusions: This is a descriptive study evaluating subjects with HCC and infected with HCV in relation to HCV treatment and fibrosis stage. Treatment of HCV did not remove the risk of development of HCC in a subgroup of patients with liver cirrhosis. Development of HCC in the absence of HCV eradication occurred in a sizeable proportion of subjects despite lower stages of fibrosis. Both findings suggest that additional risk factors for HCC development persisting after successful HCV eradication and/or prior to development of advanced fibrosis warrant investigation.

P0367

SARCOPENIA IS ASSOCIATED WITH A REDUCED SURVIVAL IN PATIENTS WITH HEPATOCARCINOMA UNDERGOING SORAFENIB TREATMENT

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Background and Aims: Sarcopenia, a condition characterized by muscle wasting, has been associated with poor outcomes in patients with cirrhosis and solid tumors, and with a higher toxicity rate during chemotherapy. Hepatocarcinoma (HCC), presently the third cause of cancer death worldwide, is diagnosed at advanced stage in up to 40% of all patients, and at this stage, the only approved treatment is sorafenib. We analyzed the influence of sarcopenia on the survival of HCC patients treated with sorafenib.

Methods: We conducted a retrospective study on 41 patients affected by advanced HCC treated with sorafenib. Patients who had performed an abdominal computed tomography (CT) 30 days within treatment start were enrolled, and data regarding survival, treatment toxicity, pre-treatment anthropometric features, and laboratory findings were collected. A transverse CT image with a multiplanar reconstruction (MPR) from L3 was collected from each scan and analyzed with SliceOmatic V 5.0, a software enabling specific tissue selection. The muscles' cross-sectional area at this level were selected and normalized for height, obtaining the skeletal muscle index (SMI). Presence of sarcopenia was defined by the presence of a SMI ≤41 cm^2/m^2 for women, and ≤53 cm^2/m^2 for men with a body mass index (BMI) ≥25 kg/m^2 and ≤43 cm^2/m^2 for those with a BMI <25 kg/m^2.

Results: Patients were mainly males (80.5%). Sarcopenia was present in more than one third of the patients (36.5%, 15/41), with no difference in sex distribution (M 30.3% vs F 62.5% p = 0.094). The two groups, with or without sarcopenia were compared. Baselines features were similar, no significant difference in albumin levels, international normalized ratio, BMI, baseline serum sodium, creatinine, bilirubin and MELD score were detected. Child–Pugh Score was significantly higher in the sarcopenic group [6 (5–8) vs 5 (5–8); p = 0.034]. Age was also slightly higher in the sarcopenic group: 74 (31–85) vs 64.5 (50–80) years, p = 0.043. Patients with sarcopenia had a reduced Overall Survival, 79 (95% CI 44–114) vs 181 (95% CI 140–223) weeks (p = 0,0013), as well as smaller survival after treatment of 24 vs 54 weeks (Figure 1). Time on treatment of sarcopenic patients was 12.3 vs 22.9 weeks (HR 1.83, Logrank test p = 0.0464), but there were no significant differences in the cause of interruption between the two groups.

Conclusions: Sarcopenia is present in a third of advanced HCC, and it is associated with a reduced OS as well as with reduction of the time on sorafenib treatment.

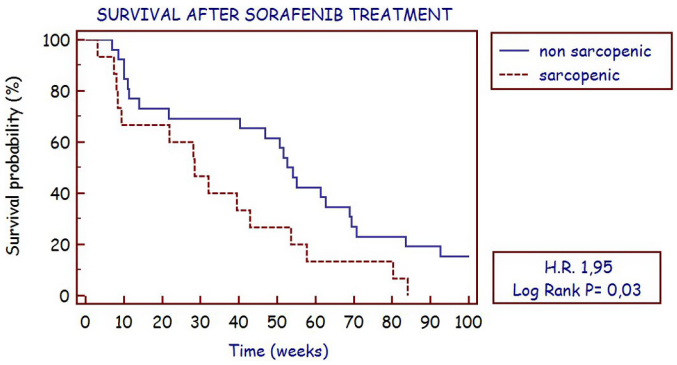

Figure 1.

P0368

LONG TERM SURVIVAL AFTER REPEAT LIVER RESECTION FOR COLORECTAL LIVER METASTASIS IS POSSIBLE

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Background and Aims: More than 50% of patients with colorectal cancer will develop liver metastases (CRLM). The surgical resection is the standardized treatment of choice and the only potentially curative, aided by chemotherapy.
Nevertheless 40% of the patients will have recurrence in the liver after the first operation, and 20–40% will be potential candidates to repeat hepatectomies.
The aim of our study was to evaluate the difference between first, second and third operation for CRLM in term of long term survival, tumour characteristics and intraoperative data.

Methods: A retrospective study of patients undergoing repeat hepatectomies at The Queen Elizabeth Hospital in Adelaide between 1997 and 2013 was performed.
In total 415 patients have been registered in our Liver Database between 1997 and 2013, 225 had CRLM.
We included a total of 37 patients who had a second liver resection for CRLM and 6 who had a third resection. The control group comprised the 182 patients who had only first resection.
Data collected concerned primary tumour and metastases' characteristics, liver resection, perioperative treatment, recurrence and survival. Data about recurrence included site, date of appearance, and treatment. The overall survival after repeat resection was determined and compared with that of patients underwent only first liver resection during the same period.

Results: The median time of follow-up was 35.4 months (range 1.43 and 210.46), 3 patients were lost to the follow-up. The median time between primary bowel resection and first liver resection was 274 days (range 29–1725), 344 days (range 35–1639) between first and second and 531 days (range 327–1161) between second and third resection.
The median disease free survival time was 32.61 months (range 1.43–210.46). The t student test on duration of the surgery and blood loss didn't show statistical differences in term of surgical difficulty between the three groups.
One patient who had a second hepatectomy died for MRSA sepsis.
All the other patients did not present perioperative mortality and major complications (Clavien grades III-IV).
The 1–3-5 and 10 year survival rates were respectively 89.2%, 47.7%, 30.4% and 20.2% after the second resection, and after the third resection were 83.3%, 50%, 33.3% and 33.3% respectively at 1–3-5 and 10 years. The comparison between those and the group of only one hepatectomy didn't show any difference.

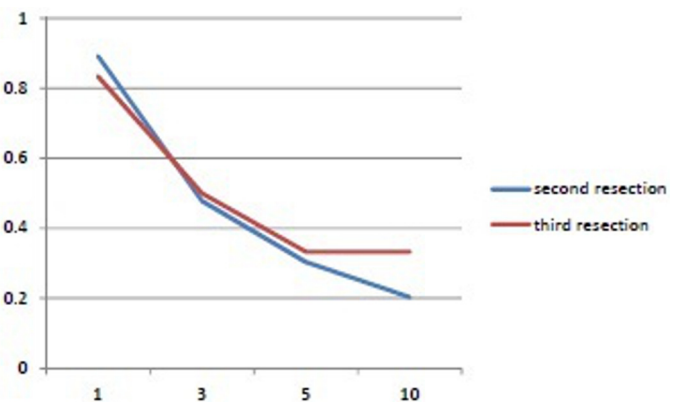

Figure 1. Comparison of survival between patients who had 2 resections and patients who had 3 resections (Kaplan–Meier test).

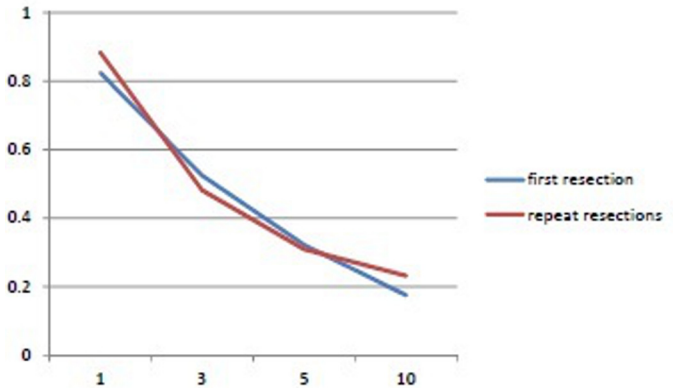

Figure 2. Comparison of survival between patients who had only 1 liver resection for colorectal metastases and patients who had repeat resections.

Conclusions: Definitively repeat resections are safe and improve long term survival.

P0369

CIRCULATING SCCA–IgM COMPLEX IS A USEFUL BIOMARKER TO PREDICT THE OUTCOME OF THERAPY IN HCC PATIENTS

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Background and Aims: Every year HCC develops in about 3–4% of cirrhotic patients. The squamous cell carcinoma antigen (SCCA) was found elevated in liver cancer specimens by immunohistochemistry and detected by ELISA complexed with IgM (SCCA–IgM) in serum of patients with HCC. This study aimed to evaluate the ability of SCCA–IgM serum levels to predict the efficacy of HCC therapy.
Methods: From April 2012 to April 2014, 218 patients with a new diagnosis of HCC were enrolled in a prospective study. The diagnosis of HCC was made according to the AASLD 2010 guidelines. The patients were staged and treated according to BCLC Staging System; in particular, BCLC stage A and B were treated with loco-regional therapy (RF, LTA, PEI, TACE), and BCLC stage C were treated with Sorafenib. Response to therapy was evaluated with imaging techniques according to the mRECIST criteria. Serum SCCA–IgM levels were determined by the Hepa-IC kit (Xeptagen SpA, Venezia, Italy) at basal time. The quantization of the complex SCCA–IgM was expressed in Arbitrary Units (AU/mL).
Results: At the time of diagnosis, SCCA–IgM was reactive in 168/218 patients (77%), mean±SD: 274.4±263.2 AU/mL. In particular SCCA–IgM was detected in 95/121 (78%) patients with BCLC A and B, and 73/97 (75%) patients with BCLC C, with the mean±SD value of 258.7±280.5 AU/mL and 295.1±242.5 AU/mL, respectively. According to mRECIST criteria, 131/168 (78%) patients showed a positive response to therapy; in particular, 83/95 (87%) of BCLC A and B patients, and 48/73 (66%) of BCLC C patients. In non-responder patients (37/168) levels of SCCA–IgM (median: 165.1 AU/mL, range: 116.8–239.1 AU/mL) were significantly higher than levels measured in patients with partial, complete or stationary response to treatment (median: 126.7 AU/mL, range: 50.0–226.9 AU/mL) (Mann Whitney U Test $p < 0.05$).
Conclusions: These results suggest that the determination of SCCA–IgM complex may be helpful in predicting the response to therapy in patients with HCC.

P0370

ROLE OF GADOXETIC ACID-ENHANCED MAGNETIC RESONANCE IMAGING IN THE LONG-TERM FOLLOW-UP OF SMALL EQUIVOCAL HEPATIC LESIONS IN PATIENTS WITH COLORECTAL CANCER

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Background and Aims: The initial abdominal computed tomography (CT) scans of patients with colorectal cancer sometimes reveal equivocal hepatic lesions. We evaluated the outcomes of equivocal hepatic lesions found by CT and the diagnostic accuracy of subsequent liver MRI.
Methods: Between January 1, 2009 and December 31, 2009, equivocal hepatic lesions of <1 cm in size on preoperative CT scans in patients who underwent colorectal cancer resection were included. Gadoxetic acid-enhanced liver MRI was subsequently performed in all patients.
Results: Overall, 121 equivocal hepatic lesions were detected on preoperative CT in 65 out of 494 patients (13.2%) who underwent colorectal surgery. Based on subsequent MRI, 11 lesions were classified as definitive metastatic lesions and 100 were benign lesions. Findings in the other 10 lesions were still inconclusive even after examining CT and MRI. Of the 11 lesions that were classified as metastatic lesion by MRI and were resected, 10 were pathologically confirmed as metastases and one lesion was a benign nodule. All 100 benign lesions were stable on follow-up imaging. Of the 10 equivocal lesions, 6 increased in size during the follow up, suggesting they were early metastases, while 4 were stable. The sensitivity and specificity for detecting liver metastases by gadoxetic acid-enhanced MRI of equivocal hepatic lesions found by CT were 100% (16/16) and 95.2% (100/105), respectively, if MRI was equivocal or indicated definite metastasis. The negative predictive value was 100% (100/100).
Conclusions: MRI is a useful diagnostic tool for assessing equivocal hepatic lesions that require further evaluation.

Liver tumors: c. Management

P0371
A MULTI-INSTITUTION PHASE II STUDY OF GEMCITABINE/CISPLATIN/S-1 (GCS) COMBINATION CHEMOTHERAPY FOR PATIENTS WITH ADVANCED BILIARY TRACT CANCER (KHBO 1002)

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Background and Aims: Gemcitabine/cisplatin combination therapy has been the standard palliative chemotherapy for patients with advanced biliary tract cancer (BTC). We aimed to evaluate the efficacy and safety of adding S-1 to gemcitabine/cisplatin combination therapy for patients with advanced BTC.
Methods: Patients with histologically or cytologically confirmed unresectable or recurrent BTC were eligible for inclusion. The primary endpoint was overall survival. Based on the results of our preceding phase I study, gemcitabine and cisplatin were administered intravenously at doses of 1,000 mg/m^2 or 25 mg/m^2, respectively, on day 1, and oral S-1 was administered daily at a dose of 80 mg/m^2 on days 1–7 every 2 weeks.
Results: Fifty patients enrolled between October 2011 and August 2012 were evaluated. After a median follow-up of 15.1 months (range, 2.4–24.4 months), the median overall survival time was 16.2 months [95% confidence interval (CI), 10.1–22.3 months], and the 1-year overall survival rate was 59.9% (95% CI, 46.2–73.5%). The grade 3–4 hematological toxicities were as follows: neutropenia (32%), anemia (32%), thrombocytopenia (10%), and febrile neutropenia (4%). The common grade 3–4 non-hematological toxicities were biliary tract infection (14%), anorexia/nausea (10%) and fatigue (8%).
Conclusions: Gemcitabine/cisplatin/S-1 combination chemotherapy offered a promising survival benefit with manageable toxicity in patients with advanced BTC. A randomized phase III trial to investigate the efficacy of this regimen compared to gemcitabine/cisplatin combination therapy in patients with advanced BTC is now underway (UMIN000014371/NCT02182778).

P0372
GIDEON: A RETROSPECTIVE ANALYSIS OF PROGNOSTIC FACTORS FOR SURVIVAL

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Background and Aims: GIDEON was a global observational study of patients with unresectable HCC treated with sorafenib, conducted in 39 countries and 5 global regions. GIDEON established a database of clinically relevant information, allowing retrospective analysis to identify prognostic factors for survival in sorafenib-treated patients.
Methods: Data were collected from >3000 unresectable HCC patients for whom the decision to treat with sorafenib had been made with a life expectancy >8 weeks. The safety population included patients with at least 1 dose of sorafenib and 1 follow-up assessment. Univariate and multivariate analyses were performed using Cox proportional hazards regression models to identify factors affecting their prognoses for survival. OS was paired with the following factors used individually as independent variables for the analyses: age; sex; region; largest tumour size; number of lesions; BCLC score; Child–Pugh status; extrahepatic spread; Child–Pugh status including ascites, bilirubin, albumin, *INR and* encephalopathy; ECOG PS; AFP; response to last previous TACE treatment; number of previous TACE treatments; hepatitis B and C; alcoholism; vascular invasion. Hazard ratios (HRs) with confidence intervals were calculated for each of the independent variables except for non-ordered categorical variables. Multivariable analyses were also performed. HRs were used to judge the strength of association between the dependent and independent variables.

Table: Results of univariate analysis

Variable	N	Hazard ratio	95% confidence interval
BCLC stage	2704	1.665	1.535, 1.807
Child–Pugh status	2717	2.114	1.924, 2.322
Total bilirubin <2, 2–3, >3	2897	1.596	1.469, 1.732
Albumin	2990	1.755	1.629, 1.892
INR <2, 2–3, >3	2408	1.040	0.872, 1.240
Ascites	3101	1.900	1.757, 2.056
Encephalopathy	3081	1.239	0.913, 1.681
ECOG PS	3018	1.680	1.573, 1.794
Extrahepatic spread	3213	1.313	1.189, 1.449
Largest tumour size	3213	1.538	1.395, 1.696
Number of lesions	2907	1.338	1.207, 1.483
Vascular invasion	3213	1.540	1.376, 1.725
Aetiology hepatitis B	3213	1.109	1.002, 1.227
Aetiology hepatitis C	3213	0.869	0.782, 0.966
Alcohol use	3213	1.138	1.032, 1.255
AFP <400, ≥400	2671	2.060	1.849, 2.296

Results: 3213 patients were included in the analyses. Prognostic values of baseline characteristics and staging systems for median

OS are shown in the table with HRs from the univariate analyses. Baseline characteristics and scoring/staging systems, including ECOG PS, Child–Pugh status and BCLC stage, appear to be prognostic factors for OS, as well as albumin, bilirubin and ascites. Encephalopathy and INR are not strong prognostic factors. Measures of extent of disease, including extrahepatic spread, larger tumour size, higher number of lesions and AFP, are prognostic factors of shorter survival time. Child–Pugh score is the most influential prognostic factor. Further analyses will be presented.
Conclusions: GIDEON data indicate that the scoring/staging systems including Child–Pugh status and BCLC stage appear to be useful prognostic factors for OS. The weight of each factor in Child–Pugh score may differ and it may be useful to find a new scoring system that can determine factors for survival in patients treated with sorafenib.

P0373
TWELVE-YEAR OUTCOMES OF RADIOFREQUENCY ABLATION AS FIRST LINE TREATMENT FOR HEPATOCELLULAR CARCINOMA IN MILAN CRITERIA: ANALYSIS OF 804 PATIENTS IN A SINGLE CENTER

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Background and Aims: Radiofrequency ablation (RFA) has been widely performed for treatment of early hepatocellular carcinoma (HCC) as a curative treatment with advantage of minimal invasiveness and safety. Many studies have confirmed the safety and efficacy of RFA for HCC and reported excellent long-term prognosis. The aim of this study is to evaluate 12-year outcomes of RFA as an initial therapy for early HCC in a single center.
Methods: From Nov 2001 to Dec 2013, 804 patients who diagnosed as early HCC (total 804, mean size: 2.1 cm) were treated with percutaneous RFA as an initial option. The mean follow up time was 38.1 months (range: 0–151 months). RFA was performed with ultrasound-guidance to single nodular HCC, less than 5 cm in maximum diameter or multiple (up to 3) nodular HCCs, each diameter should be under 3 cm in maximum. Overall survival and disease free survival rates were estimated by the Kaplan–Meier method and the effect of risk factors on recurrence was assessed by the Cox proportional hazard model.
Results: The study population showed male dominance (male: n = 608, female: n = 196) and mean age was 60.3 years, ranging from 24 to 86 years old. Patients had better Child–Pugh class (A: n = 710, B: n = 93, C: n = 1) and AFP was elevated. (mean: 295.1, range: 0.7–42,690 ng/mL). Treatment failure occurred in 5 patients as mistargeting and insufficient coverage. Cumulative overall survival rates at 1-, 3-, 5-, 10-, 12-year were 92.9%, 83.4%, 59.4%, 42.8%, and 37.5% respectively. Cumulative disease free survival rates at 1-, 3-, 5-, 10-, 12-year were 75.7, 39.0%, 23.1%, 9.8%, and 4.9% respectively. Risk factors for tumor recurrence were age (HR = 1.334; 95% CI 1.102–1.613), tumor number (HR = 1.392; 95% CI 1.149–1.687) and size (HR = 1.260; 95% CI 1.103–1.440), AFP level (>200 ng/mL, HR = 1.324; 95% CI 1.026–1.708), and Child–Pugh score (HR = 2.132; 95% CI 1.656–2.745).
Conclusions: We analyzed the treatment outcome of RFA as first line treatment for early HCC, which showed good results of overall survival and disease free survival. Risk factors for recurrence were age, tumor number and size, AFP level, and Child–Pugh score.

P0374
PROGNOSIS OF EARLY-STAGE HEPATOCELLULAR CARCINOMA: THE CLINICAL IMPLICATION OF SUB-STAGES OF BARCELONA CLINIC LIVER CANCER SYSTEM

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Background and Aims: Curative therapies are proposed for early-stage hepatocellular carcinoma (HCC) according to the Barcelona Clinic Liver Cancer (BCLC) staging system. We aimed to compare the prognoses between HCC patients in different sub-stages of BCLC stage 0 or A.
Methods: We enrolled 1266 treatment-naïve HCC patients with BCLC stage 0 or A at Taipei Veterans General Hospital from October 2007 to April 2014. Patients were stratified into five (0, A1–A4) sub-stages and factors in terms of overall survival and recurrence were analyzed. Hepatic resection surgery and radiofrequency ablation (RFA) were defined as curative treatment, while transarterial chemoembolization, best supportive treatment, systemic chemotherapy, sorafenib, radiotherapy, and chemo-radiotherapy were regarded as non-curative treatment.
Results: There were 184, 446, 272, 92 and 272 patients in sub-stage 0, A1, A2, A3 and A4, respectively. After a median follow up period of 69.4 months, the cumulative 5-year overall survival rates in these sub-stages were 72.1%, 65.8%, 48.8%, 33.2% and 46.0%, respectively ($p < 0.001$). Patients in the BCLC stage 0 and A1 had comparable prognoses (p = 0.136), and both of them had significantly higher overall survival rates than those in stage A2–A4 (all $p < 0.001$). However, there were no significant differences in overall survival between BCLC stage A2, A3 and A4. Multivariate analysis revealed that factors associated with overall mortality were serum albumin ≤3.5 g/dL ($p = 0.009$), alpha-fetoprotein (AFP) >20 ng/ml ($p < 0.001$), tumor size >3 cm ($p = 0.003$), BCLC stage A2–A4 (p = 0.013), and non-curative treatment (p < 0.001). Besides, there were 1012 patients who underwent curative treatments, including 480 patients with hepatic resection and 532 patients with RFA. Multivariate analysis disclosed that positive for anti-hepatitis C virus (p = 0.010), alanine aminotransferase >40 U/L (p = 0.001), AFP >20 ng/ml (p = 0.005), BCLC stage A2–A4 (p = 0.003), and RFA (p = 0.019) predicted higher incidence of developing recurrence.
Conclusions: The sub-stages of BCLC staging system provided good prognostic stratification for early-stage HCC. Patients with single tumor larger than 2 cm but without significant portal hypertension or jaundice had similar prognosis to those in BCLC stage 0. Moreover, treatment modality was crucial in determining the prognosis of early-stage HCC patients. Curative therapies, especially hepatic resection, should be performed in early-stage HCC.

P0375
THE VALIDATION OF HONG KONG LIVER CANCER STAGING SYSTEM AND COMPARISION WITH BARCELONA CLINIC LIVER CANCER FOR PREDICTION OF SURVIVAL AND TREATMENT IN HEPATOCELLULAR CARCINOMA PATIENTS

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Background and Aims: In addition to world widely endorsed BCLC system, various algorithms and staging systems were developed in

many organizations, including the Hong Kong Liver Cancer (HKLC) staging. We validated the HKLC system and compared it with BCLC in HCC patients.
Methods: The medical records of 875 HCC patients from 2004 to 2009 were retrospectively reviewed. The data including performance status, Child–Pugh score, tumor characteristics, treatment modality and survival were collected.
Results: The etiology was predominantly chronic hepatitis B (CHB) (71.2%) and 90% of patients had child A and B liver function. Median follow up duration was 21.7 (0–178) months. Seventy-five percent of patients died during study period and median overall survival was 22.6 (0–181) months. The median overall survival was 45.44, 25.25, 21.25, 6.58, 3.19, 3.50, 3.00, 13.50 and 1.95 months in HKLC stage I, IIa, IIb, IIIa, IIIb, IVa, IVb, Va and Vb, respectively. Both HKLC and BCLC well differentiated the survival ($p < 0.001$). However, HKLC significantly well predicted 1 and 2 year of survival than BCLC (AUROC; 0.833 vs 0.801, $p = 0.0027$; 0.826 versus 0.799 in 2 year, $p = 0.0144$). In the patients in BCLC B and HKLC II, the curative therapy group recommended by HKLC showed better survival compared to TACE which recommended by BCLC (median overall survival; 55.57 vs. 28.22 months, $p = 0.013$). In BCLC B, the patients who treated according to HKLC showed better survival than who treated by BCLC (median overall survival; 45.34 vs. 31.00 months, $p = 0.004$). In BCLC subclass B1, the curative therapy was superior to TACE (median overall survival; 55.57 vs. 28.12 months, $p = 0.038$), while the differences were not significant in BCLC B2 and B3. In the patients in BCLC C and HKLC II, the curative therapy group recommended by HKLC showed better survival compared to systemic therapy which recommended by BCLC (median overall survival; 51.10 vs. 6.57 months, $p < 0.001$). Also, in BCLC C and HKLC III, the TACE group recommended by HKLC showed better survival compared to systemic therapy which recommended by BCLC (median overall survival; 15.78 vs. 5.87 months, $p = 0.007$).
Conclusions: The HKLC system showed better survival compared to BCLC system in our population. The more individualized therapy could be considered by HKLC for management of HCC patients to improve prognosis.

P0376
STRATIFICATION OF HEPATOCELLULAR CARCINOMA. THE PROGNOSTIC SCORE NIACE, AN ADDITIONAL AID TO THE BARCELONA CLINIC LIVER CANCER (BCLC) STAGING SYSTEM? MULTICENTER STUDY

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Background and Aims: NIACE score: 1×(Nodules 0 if <3, 1 if ≥3) + 1.5×(Infiltrating 0 if no, 1 if yes) + 1.5×(AFP: 0 if <200, 1 if ≥200 ng/ml) + 1.5×(Child–Pugh 0 if A, 1 if B) + 1.5×(ECOG PS 0 if 0, 1 if ≥1), distinguishes 2 groups with different survival [3 external validation cohorts of 543 BCLC C patients (pts)], regardless of treatment (poster session 1329 AASLD 2014).
To confirm the prognostic value of NIACE score in BCLC A, BCLC B HCC pts, regardless of treatment (excluded transplantation). To assess the NIACE score in HCC pts treated by transarterial chemoembolization (TACE) or Sorafenib.
Methods: Were selected over a period of 5 years (Marseille, Nancy, Rennes, Bordeaux): BCLC A pts n = 128, BCLC B pts n = 195; pts treated by TACE n = 230; BCLC C pts treated by Sorafenib n = 285. Evaluation of NIACE score on the entire cohort.
Results: In BCLC A pts NIACE distinguished with a threshold value ≤1, 2 groups with a different survival: NIACE ≤1 n = 85, 38 months [35–51] vs. >1 n = 43, 24 months [19–35] $p = 0.0042$.
In BCLC B pts, NIACE distinguished with a threshold value ≤2.5, 2 groups with a different survival: Marseille NIACE ≤2.5 n = 73, 22 months [18–27] vs. >2.5 n = 31, 13 months [9–16] $p = 0.0002$; Nancy NIACE ≤2.5 n = 85, 27 months [23–36] vs. >2.5 n = 6, 13 months [13–23] $p = 0.0015$.
NIACE score applied to HCC pts (BCLC A, B, C) treated by TACE, distinguished with a threshold value ≤2.5, 2 groups with a different survival: Marseille NIACE ≤2.5 n = 160, 28 months [26–35] vs. >2.5 n = 70, 9.6 months [7–12] $p < 0.0001$.
NIACE score applied to HCC pts (BCLC C) treated by Sorafenib, discerned with a threshold value <3, 2 groups with a different survival: Nancy NIACE <3 n = 28, 16 months [14–25] vs. ≥3 n = 55, 6 months [4–8] $p < 0.0001$; Rennes NIACE <3 n = 37, 10.6 months [4.1–17.1] vs. ≥3 n = 46, 5.1 months [2.9–7.4] $p < 0.0001$; Bordeaux NIACE <3 n = 44, 11.3 months [8.1–15.9] vs. ≥3 n = 75, 6 months [4.3–7.6] $p < 0.0001$.
Whatever the stage, the type of treatment, the probability of survival were significantly different between the different score's value and the score variation was correlated with the survival.
Conclusions: This study suggests the ability of NIACE score to discern among BCLC A, B, C HCC subgroups with different prognosis. This score may be also an aid to the decision making process, because it discerns among patients treated by TACE or Sorafenib, subgroups with different prognosis.
The NIACE score should be assessed in prospective studies to confirm its prognostic value and permit a sub classification of HCC useful for clinicians

P0377
TUMOR NUMBER IMPROVES THE PROGNOSTIC VALUE OF HEPATOMA ARTERIAL-EMBOLIZATION SCORE IN PATIENTS WITH HEPATOCELLULAR CARCINOMA TREATED WITH CHEMOEMBOLIZATION

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Background and Aims: The hepatoma arterial-embolization prognostic (HAP) score predicts survival outcome in patients with hepatocellular carcinoma (HCC) undergoing transarterial chemoembolization (TACE). We tried to validate HAP score in Korean subjects with HCC and investigated whether the prognostic performance of HAP score can be improved using additional clinical parameters.
Methods: A total of 280 patients with unresectable HCC treated with TACE between January 2003 and December 2009 were included. Validation and subsequent modification of HAP score was performed based on multivariate Cox regression models for overall survival (OS).
Results: The mean age of the study population (men 211 and women 69) was 59 years. Viral etiology of HCC accounted for 80.4% (n = 181 and 44 for hepatitis B and C virus). The median OS was 40.5 (95% confidence interval 34.0–47.0) months. On multivariate analysis, together with the original components of HAP score (serum albumin <3.6 g/dL, total bilirubin >0.9 mg/dL, alpha-fetoprotein >400 ng/mL, and tumor size >7 cm), tumor number ≥2 was selected as an independent unfavorable prognostic factor for OS (hazard ratio 2.3; $P < 0.001$). Accordingly, a modified HAP score by adding tumor number (HAPN) was established. Although both HAP and HAPN score discriminated the four different risk groups (log-rank test $P < 0.001$), HAPN score performed significantly better

than HAP score: areas under receiver operating curves for OS at 1 year (0.763 vs. 0.733), 3 years (0.717 vs. 0.658), and 5 years (0.728 vs. 0.645), respectively (all P < 0.05).
Conclusions: HAP score accurately predicted OS for Korean subjects with HCC undergoing TACE. However, addition of tumor number to the HAP score (HAPN score) improved the prognostic performance significantly. HAPN score might be useful in accurate prognostication and selection of optimal candidates for TACE.

P0378
TIME TO PROGRESSION OF AFP IS AN INDEPENDENT PROGNOSTIC FACTOR OF SURVIVAL IN HEPATOCELLULAR CARCINOMA TREATED WITH SORAFENIB. INTERNAL VALIDATION

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Background and Aims: Previously (JCT, 2014) we have showed that *time to progression of AFP* (TPA) is an independent indicator of overall survival (OS) in hepatocellular carcinoma (HCC) treated with sorafenib (SOR), just considering baseline characteristics. TPA was defined as the period of time since beginning of SOR until the moment in which AFP increases >20 ng/mL or it starts to rise from the nadir. The greater the TPA the greater is the survival. In this study we expand and refine the previous cohort and consider other factors that appear over time, in order to asses if TPA remains useful.
Methods: Unicentric prospective cohort recruited from JUL-07 to FEB-14, followed up until 23 October 2014. Patients without cirrhosis, ECOG-PS >2 and Child >7 (n = 29) were excluded. SOR was administered at the maximum tolerated dose until clinical progression, patient desire or serious adverse event. First radiologic control made at week 12, then every 16w. We analyzed baseline characteristics, side effects, TPA at day 90, time to radiologic progression and dose modifications.
Results: 121 consecutive patients were included: Child A–B7 (106–15), ECOG PS 0–1 (92–29); 36 BCLC-B, 85 BCLC-C. Basal AFP 45.8 ng/mL. Median duration of SOR was 8 months (P_{25}–P_{75}: 4–26). Median OS since the beginning of SOR to 2nd line therapy (n = 4), death (n = 92) or last visit was 12 months (P_{25}–P_{75}: 6–30).
Predictors of OS in univariate analysis: age (p = 0.003); symptoms [ECOG (0 vs 1): 18 vs 5 months; p < 0.001]; Child–Pugh [A vs B: 16 vs 8 months; p = 0.019]; BCLC [B vs C: 28 vs 10 months; p < 0.001]; hypertension and dermatological effects are associated with increased OS but p = 0.202, p = 0.357, respectively. The maximum tolerated dose (p = 0.037), duration of treatment (p < 0.001) and time to radiologic progression (p < 0.0001) are also associated with increased OS. Finally, patients with TPA >90 days have significantly higher OS than those with TPA ≤90 [23 months (95% CI: 14–32) vs 9 months (95% CI: 7–11), p = 0.0002].
Predictors of overall survival in the multivariate analysis: time to radiologic progression [p = 0.026], duration of SOR [p = 0.023], baseline ECOG PS [p = 0.001; HR 0.397 (95% CI: 0.234–0.673)] and TPA [p = 0.002; HR 2.144 (95% CI: 1.315–3.495)].
Conclusions: TPA maintains its prognostic value in patients with HCC treated with SOR, regardless of other baseline or time-dependent covariates. TPA can be a useful tool to help in the decision of passing to second-line treatment. External validation of this marker is warranted.

P0379
CANCER RELATED SYMPTOMS (PERFORMANCE STATUS 0 VS 1) DETERMINE PROGNOSIS OF HEPATOCELLULAR CARCINOMA PATIENTS TREATED WITH SORAFENIB. ANALYSIS OF A PROSPECTIVE DATA COLLECTION IN 207 PATIENTS

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Background and Aims: *Background:* All the studies that analyze sorafenib in hepatocellular carcinoma (HCC) include a wide spectrum of patients and the majority neglect the impact of the performance status (PS) for the prediction and analysis of overall survival (OS). *Aim:* To analyze the impact of baseline performance status (PS) and Child–Pugh score (CP) on the OS.
Methods: We analyzed 2 independent prospective cohorts of HCC patients treated with sorafenib between March 2008 and July 2011 [training cohort (TC) from the BCLC (n = 143) and validation cohort (VC) from Asturias (n = 64)]. Patients with PS >1 and Child–Pugh >7, encephalopathy and clinical ascites were excluded.
Results: Table 1 shows the baseline characteristics. The median treatment duration and OS were 6.7/11.7 months in the TC and 8.2/16.4 months in VC. PS was the only independent predictor of OS in both cohorts [TC: PS 0 vs. PS 1, HR 1.86 (1.12–3.1), p = 0.017; VC: HR 2.97 (1.73–5.11), p < 0.001]. Thereby, median OS according to PS was: PS 0 = 13.1 vs PS 1 = 5.4 months; p < 0.001 in the TC, and PS 0 = 21.1 months vs PS 1 = 5.9 months; p = 0.001 in the VC. Tumor burden, biochemistry parameters including AFP and CP stage were not significant. Median OS of CPA and CPB were not statically different [CP A 12.1 (n = 121)/B7 10.9 months (n = 22), p = 0.215 in TC; and CP A 16.9 (n = 60)/B7 3.9 months (n = 4), p = 0.411 in VC]. The impact of PS was maintained irrespective of BCLC-C stage. In the TC the median OS of "BCLC-C, CP A, PS 0" vs. "BCLC-C, Child–Pugh A, PS 1" was 9.9 vs. 5.4 months for "BCLC-C, CPB 7, PS 0" and 17.9 vs. 5.9 months for "BCLC-C, CP 7, PS 1". Presence of image detected ascites at baseline did not determine a different OS (p = 0.593).

Table 1.

Baseline characteristic	Training cohort (BCLC), n = 143	Validation cohort (HUCA), n = 64
Age, median [range]	64.13 [35–80]	64.81 [27–80]
Male/Female, n	122/21	50/14
HCV/Ethanol/HVB/others, n	83/39/14/7	25/26/5/8
Child–Pugh A/B, n	121/22	60/4
Performance status, 0/1, n	121/22	45/19
BCLC stage, B/C, n	73/70	18/46

Conclusions: The differentiation between PS0 and PS1 is key to predict the prognosis of patients with preserved liver function who initiate sorafenib treatment and to analyse their outcome. Differentiation between Child–Pugh score A and B7 does not have predictive capacity and thus, lacks adequate stratification capacity in this population.

P0380
NEW RECOGNIZATION OF THE NATURAL HISTORY AND GROWTH PATTERN OF HEPATIC HEMANGIOMA IN ADULTS

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Background and Aims: Treatment indications for hepatic hemangioma are still not clearly defined due to limited and inconsistent data that are available on the natural history of hepatic

hemangiomas and on their tendency to increase in size or to cause complications. This prospective cohort study was design to investigate the natural history and growth pattern of hepatic hemangioma in adults.
Methods: From April 2010 to March 2013, adult patients with hepatic hemangioma were screened and those who had no prior treatment to the hemangioma were enrolled. A routine follow-up was performed to observe the natural history and the tendency to cause complications of the lesions.
Results: A total of 236 patients were enrolled in the study. The median size of hemangiomas was 4.5 cm (range 0.6–19.2 cm). During a median follow-up period of 48 months (range 3–266 months), 61.0% patients had hemangiomas increased in size, 23.7% patients had stable lesions, and 8.5% patients had hemangiomas decreased in size. The peak growth period of hemangiomas was in patients <30 years age (0.46±0.41 cm per year) and the growth rate decreased significantly after 50 years of age (0.21±0.40 cm per year). Hemangiomas with size <2 cm had the lowest growth rate (0.16±0.42 cm per year). The peak growth rate of hemangiomas size was 8–10 cm (0.80±0.62 cm per year), then decreased rapidly to 0.47±0.91 cm per year while the hemangiomas >10 cm. Only 9 patients had severe symptoms caused by hepatic hemangioma, of which 3 were safely managed by observation. No patients presented with hemangioma-related complications.
Conclusions: Majority of hepatic hemangiomas have the tendency to increase in size but rarely cause complications. All the hemangiomas can be safely managed by observation, and no preventive treatment is required regardless of hemangioma size.
Chinese Clinical Trial Registry, number ChiCTR-OCS-11001298.

P0381
CLINICAL IMPACT OF ACHIEVING SUSTAINED VIROLOGICAL RESPONSE IN HEPATITIS C VIRUS-INFECTED PATIENTS WITH EARLY STAGE HEPATOCELLULAR CARCINOMA

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Background and Aims: Because novel direct-acting antivirals against hepatitis C virus (HCV) improve sustained virological response (SVR) rates, the number of SVR patients with advanced fibrosis has been increasing. Therefore, the number of patients who develop hepatocellular carcinoma (HCC) after achieving SVR is expected to increase. We analyzed the clinical impact of SVR in patients with early stage HCC.
Methods: The cohort, comprising 679 early stage HCC patients with positive HCV antibodies, received an initial radiofrequency ablation (RFA) at our hospital. The cumulative recurrence rates of HCC, the dropout from the Milan criteria, and the survival rates were assessed and compared between SVR patients and non-SVR patients. Those patients who achieved SVR before initial RFA were defined as SVR patients.
Results: Between April 1997 and October 2014, 679 patients [mean age 70 years; males/females (M/F) 371/308; Child–Pugh class A/B/C 618/61/0] received RFA as an initial therapy. Overall, the cumulative survival rates at 1, 3, 5, 7, and 10 years were 97%, 89%, 70%, 55%, and 38%, respectively. The cumulative dropout rates from the Milan criteria at 1, 3, and 5 years were 13%, 41%, and 57%, respectively. The Child–Pugh scores of SVR patients (n = 32) were significantly lower than those of the non-SVR patients, and the cumulative survival rates were significantly higher than those of the non-SVR patients (5-year survival rate 76% vs 70%, p = 0.028). There was no significant difference in the cumulative recurrence rates of HCC between the SVR and non-SVR patients. Furthermore, to exclude the influence of liver function, we analyzed these rates in patients with a Child–Pugh score of 5 points. In patients with this score at the initial RFA, there was no significant difference in the cumulative survival rates, the recurrence rates, and the dropout from the Milan criteria between the SVR patients (n = 25) and non-SVR patients (n = 193).
Conclusions: The recurrence rates and the dropout from the Milan criteria in patients who developed HCC after achieving SVR were not low. In patients with a Child–Pugh score of 5 points, the overall survival was similar between the SVR and non-SVR patients. New agents that prevent the recurrence of HCC are necessary for patients who develop HCC after achieving SVR.

P0382
ANGIOTENSIN II TYPE 1 RECEPTOR ANTAGONISTS AS ADJUVANT THERAPY FOR HEPATOCELLULAR CARCINOMA PATIENTS TREATED WITH RADIOFREQUENCY ABLATION

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Background and Aims: Preliminary data report beneficial effects of inhibitors of angiotensin II converting enzyme (ACE I) in decreasing hepatocellular carcinoma (HCC) recurrence after radical therapies. However, ACE I treatment was effective only in combination to other drugs and did not seem to improve significantly overall survival (OS). On the other hand, data on the adjuvant role of angiotensin II receptor 1 antagonists (sartans) are still lacking.
Aim of this study was to evaluate whether sartan therapy administered to hypertensive patients is associated to better outcomes in HCCs treated with radiofrequency ablation (RFA).
Methods: Data on 153 patients treated with RFA were reviewed. Study population was classified in three groups: patients who received neither ACE I nor sartans (Group 1), those under ACE I treatment (Group 2) and patients who received sartans (Group 3). Patients under antihypertensive therapy had been given sartans or ACE I for at least two years before RFA. Overall survival (OS) and time to recurrence (TTR) were analyzed by means of Kaplan–Meier analysis and compared with log-rank test. Antihypertensive therapy was entered in a Cox proportional model with all the main baseline clinical and tumoral parameters and tested for post-treatment outcomes in uni-multivariate setting.

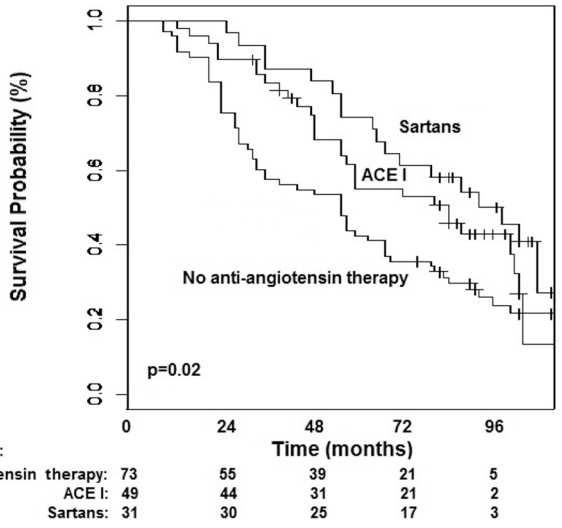

Figure 1.

Results: Group 1 consisted of 73 (47.8%) patients, while 49 (32%) and 31 (20.2%) were in group 2 and 3, respectively. In the whole study population, median age was 68 years (range 39–86), 87.8% of patients were in Child–Pugh (CP) A score and 92% in Barcelona Clinic Liver Cancer (BCLC) A stage. Median maximum diameter

was 30 mm (10–40) and alpha-fetoprotein (AFP) was 25 (1.1–2100) UI/mL. No differences in baseline characteristics among the three group were reported.
Median OS was 48 months (95% confidence interval: 31–58) in group 1, 72 months (49–89) in group 2 and 84 (58–92) in group 3 (Figure 1; p = 0.02). Median TTR was 26 (15–42), 44 (33–72) and 69 (44–74) months in the three groups, respectively (p = 0.02). Sartan therapy resulted significant predictor of longer OS (HR: 0.39, 95% CI: 0.22–0.66; p = 0.04) and delayed TTR (HR: 0.47, 95% CI: 0.27–0.82; p = 0.02) at multivariate analysis.
Conclusions: Sartan therapy given to hypertensive patients resulted effective as adjuvant treatment in HCCs treated with RFA.

P0383
COMBINED SEQUENTIAL USE OF HAP AND ART SCORES TO PREDICT TRANSARTERIAL CHEMOEMBOLIZATION FAILURE IN HEPATOCELLULAR CARCINOMA: A MULTI-CENTER COMPARATIVE STUDY

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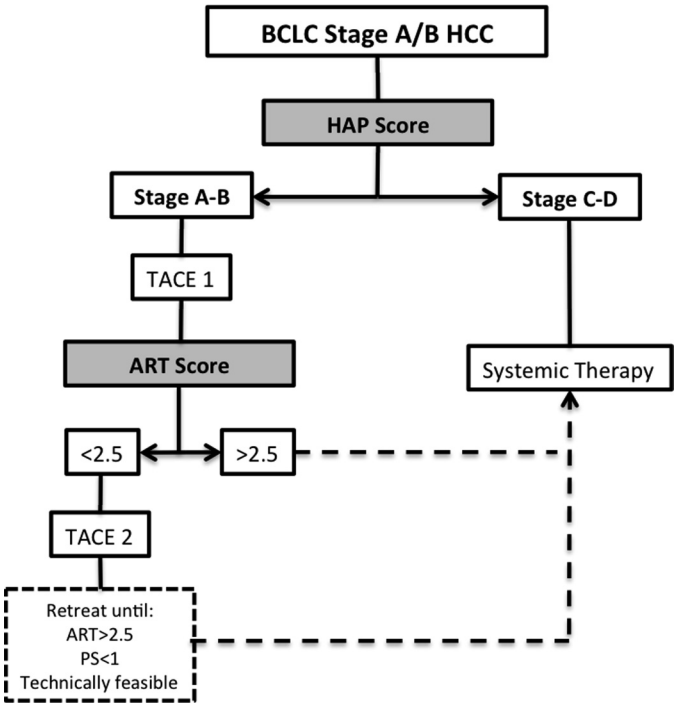
Background and Aims: The prognosis of patients with hepatocellular carcinoma (HCC) undergoing transarterial chemoembolization (TACE) is variable. We compared HAP and ART scores, established prognostic models in HCC, for accuracy of overall survival (OS) prediction.
Methods: Prognostic scores were studied in 83 consecutive unselected subjects with Barcelona Clinic Liver Cancer (BCLC) A/B stage from UK and Italy (training set) and 660 from Korea and Japan (validation set) all treated with conventional TACE using multivariate Cox regression and c-index analysis.



Results: In both training and validation sets, HAP (Hazard Ratio [HR] 3.1, 95% CI 1.8–5.3) and ART scores (HR 3.1, 95% CI 1.5–6.7) emerged as independent predictors of survival (p < 0.01) with HAP achieving better prognostic accuracy (c-index: 0.68) over ART (0.57). Subgroup analysis of BCLC-C patients receiving TACE (n = 63) revealed advanced HAP stage (p < 0.001) and radiological progression after initial TACE (p < 0.001) as adverse prognostic factors.
Conclusions: HAP and ART scores are validated indices in HCC patients undergoing initial TACE. In intermediate stage HCC, the HAP score is best suited for screening patients prior to initial TACE, whilst sequential ART assessment may improve early detection of chemoembolization failure. A separate, exploratory analysis of BCLC-C patients revealed that subjects with advanced HAP and early progression after initial TACE should not be considered for repeat TACE. We propose sequential integration of HAP and ART (Figure 1) to optimise TACE administration in patients within BCLC-B criteria.

P0384
ASSESSMENT OF TREATMENT RESPONSE BY SEQUENTIAL SERUM METABOLOMIC PROFILING AFTER PERCUTANEOUS RADIOFREQUENCY ABLATION OF HEPATOCELLULAR CARCINOMA

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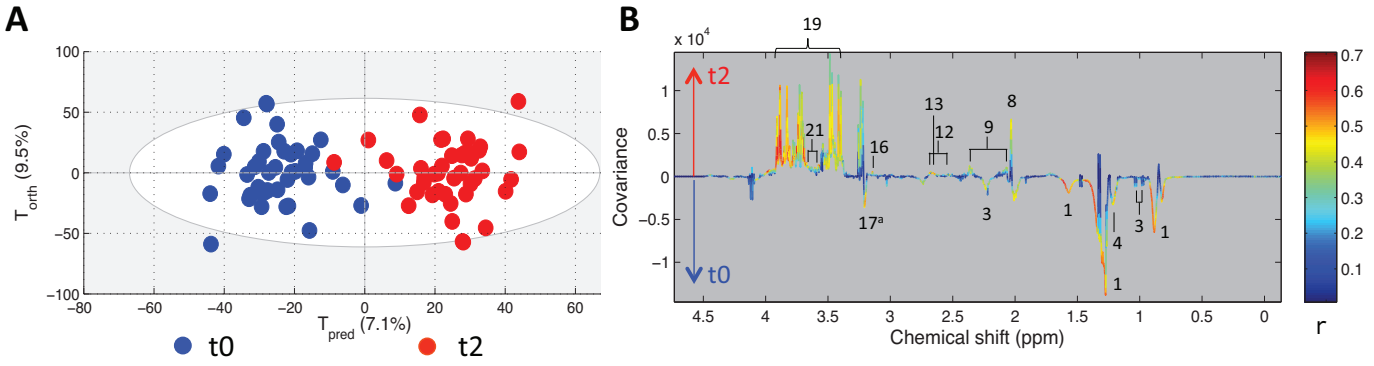
Background and Aims: Radiofrequency Ablation (RFA) is commonly performed as a curative approach in patients with HCC. However, the risk of tumour recurrence is difficult to predict due to a lack of viable clinical and biological markers. Metabolomics appears to be a promising technique leading to the identification of new biomarkers. The aim of this work was to assess changes in serum metabolomic profiling of patients treated by RFA and to study their potential ability to predict tumour recurrence.
Methods: Sequential sera obtained before and after RFA procedure in 120 patients with HCC fulfilling Milan criteria developed in cirrhosis were investigated using Nuclear Magnetic Resonance (NMR) metabolomics. Sequential sera samples were drawn at 3 different points (before RFA, one day post procedure and at evaluation of efficacy within 3 months after RFA). With the combination of classical multivariate data analysis such as Principal Component Analysis (PCA) and Orthogonal Partial Latent Structure (OPLS), a multilevel OPLS analysis was also used in order to discriminate the metabolic intra-individual changes caused by the RFA intervention. Patients were prospectively followed-up and recurrence-free survival was studied.
Results: A specific serum metabolic fingerprint of the RFA procedure could be identified. Major changes of metabolites in the sera linked to the RFA intervention included an increase in the concentration of lactate, glutamine, 3-phenylpropionate and a decrease in the concentration of isoleucine, proline, N-acetyl functions of glycoproteins and phosphocholine-glycerophosphocholine (PC-GPC) post-RFA. Furthermore, two distinct serum metabolic profiles of HCC patients were identified according to viral or non-viral aetiology of liver disease, this distinction being further observed with specific and distinct metabolic responses after RFA procedure. During a median follow-up of 55 months, 61 (48%) patients experienced HCC recurrence. Among the viral-related HCC patients, those who were the better discriminated by the metabolomic profiles presented a



	Metabolite	Chemical shift (ppm) and multiplicity°	r*
1	Lipids	0.81[b]; 1.25[b]; 1.55[b]	↘
3	Valine	1.04[d]; 2.25[m]	↘
4	3-hydroxybutyrate	1.19[d]; 4.16[m]	↘
8	N-acetyl glycoprotein	2.02[s]	↗
9	Glutamate	2.02[m]; 2.36[m]	↗
12	Citrate	2.52[d]; 2.69[d]	↗
13	Methionine	2.63[t]	↗
15	Malonate	3.14[s]	↗
16	τ-methylhistidine	3.14[dd]	↗
17[a]	Choline	3.19[s]; 4.07[m]	↘
19	Glucose	3.24[dd]; 3.41[t]; 3.42[t]; 3.46[m]; 3.49[t]; 3.54[dd]; 3.71[t]; 3.73[m]; 3.84[m]; 3.9[dd]; 5.23[d]	↗
21	Glycerol	3.56[dd]; 3.64[dd]	↗

Chemical shift and multiplicity correspond to those found in the ^{1}H-NMR spectra of patients' sera.
° ppm: parts per million. s, singlet; b, broad; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet.
*Trend of correlation

Figure 1 (abstract P0384). Multilevel OPLS-DA score plot (A) and loading plot (B) of the within subject variation obtained from serum viral HCC patients before RFA, t0 (blue) and after 1–4 months post-RFA, t2 (red) (42 patients). Variations of metabolites are represented using a line plot between 0–4.5 ppm and are summarized in tabular form (C). Positive signals correspond to metabolites present at increased concentrations in the taken sera 1–4 months post-ablation period (t2). Conversely, negative signals correspond to metabolites present at increased concentrations in sera taken during pre-ablation period (t0).

lower rate of recurrence (58% *vs.* 81%, $P = 0.02$) than patients with a lower discrimination between their pre-RFA and delayed samples.
Conclusions: RFA in HCC patients leads to profound changes in the serum metabolome that further seem to be specific of the aetiology of liver disease. Sequential assessment of serum metabolomic profiles during follow-up of these patients provides new biomarkers of treatment efficacy and may help to predict HCC recurrence.

P0385
PROGNOSTIC IMPACT OF THE BAVENO IV STAGING SYSTEM OF PORTAL HYPERTENSION IN PATIENTS WITH CIRRHOSIS AND HEPATOCELLULAR CARCINOMA

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Background and Aims: D'Amico et al. proposed a portal hypertension (PTH)-staging system for patients with liver cirrhosis, which was acknowledged by the Baveno IV consensus workshop on methodology of diagnosis and therapy in PHT.
Here, we investigated the prognostic impact of this PTH-staging system in patients cirrhosis and the hepatocellular carcinoma (HCC).
Methods: Patients with liver cirrhosis diagnosed with HCC between 1994 and 2012 treated with non-surgical therapies at the Medical University of Vienna were included (n = 790). The PTH staging system (stage 1: no ascites/varices, stage 2: varices/no ascites; stage 3: ascites +/− varices; stage 4: bleeding +/− ascites) and several other clinically important parameters of liver function and tumor load were entered into univariate analysis. Variables with a p-value <0.05 were included into a multivariate Cox-regression model.
Additionally, the prognostic role of beta-blocker (BB) therapy was investigated in patients with PTH-stage >1.
Results: The PTH staging system was significantly associated with overall survival (OS) on univariate analysis and remained a significant prognostic factor upon multivariate analysis independent from BCLC stage, Child–Pugh stage, CRP-levels, MELD-score and tumor load. The median OS for PTH-stage 1/2/3/4 was 22/14/4/6 months ($p < 0.001$). Of 585 patients with PTH-stage >1, only 179 received betablocker-therapy which was associated with better median OS (BB vs. no BB: 10.2 vs. 5.8 months, $p = 0.026$).
Conclusions: The Baveno IV PTH staging system was an independent prognostic factor in patients with HCC. We noticed a severe under usage of BB in patients with portal hypertension and HCC. Patients who received BB had a significant better outcome than those without BB.

P0386

PREDICTING LIVER DECOMPENSATION AFTER RESECTION FOR HEPATOCELLULAR CARCINOMA: A RECURSIVE PARTITIONING ANALYSIS OF PROGNOSTIC FACTORS

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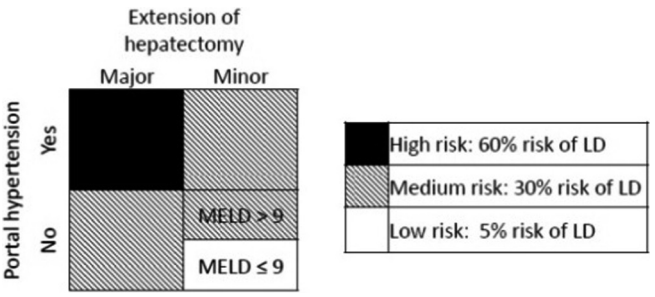
Background and Aims: Liver resection (LR) is the treatment of choice for hepatocellular carcinoma (HCC) in well compensated liver cirrhosis. Various grades of liver decompensation (LD) after LR represent an important cause of morbidity and mortality difficult to predict preoperatively. Aim of this study was to define risk classes for LD based on preoperative variables.
Methods: In a single western institution, 543 HCC patients underwent resection over a 10 years period. Postoperative complications were recorded according to Dindo–Clavien's classification (DCC). LD was defined as postoperative occurrence of any of the following conditions associated with a DCC >1: clinically detectable ascites, prothrombin time <70% or bilirubin >3 mg/dL. Pre-operatively, clinically relevant portal hypertension (PH) was defined by the presence of esophageal varices or the coexistence of low platelet count ($<100\times10^3/mm^3$) and spleen diameter >120 mm. Major resection was defined as removal of 3 or more adjacent segments. Logistic regression was performed to identify risk factors to be included in a Recursive Partitioning Analysis (RPA) model in order to identify risk classes predicting LD on the basis of preoperative conditions.
Results: Median age of the patients was 68 years (IQR 62–73). 287 were HCV (52.8%). Most patients presented a well-preserved liver function, being 93.5% in Child–Pugh A with a median MELD score of 8 (IQR 7–10); PH was present in 163 (30%) cases. BCLC staging was: 0 in 76 (14%), A in 381 (70.2%), B in 64 (11.8%), C in 22 (4%). Major resections were 105 (19.3%) and minor 438 (80.7%). LD occurred in 108 patients (20%); logistic regression identified PH, major liver resection and MELD >9 as independent predictors of LD. RPA classifications identified three risk classes: low (5%), intermediate (30%) and high (60%) risk. Low-risk (226 patients) were patients without PH aimed at minor resections with a MELD score ≤9; intermediate risk (297 patients) were patients without PH aimed at major resections or those undergoing minor resections with PH or MELD >9; high-risk (20 patients) were patients with PH undergoing major resections. Median length of stay was significantly different among the three risk classes: 7, 8 and 11 days respectively ($p<0.001$), as well as liver-related mortality: 0.5%, 9% and 25% respectively.
Conclusions: Risk of LD can be accurately stratified preoperatively according to a simple algorithm built on presence of PH, planned extension of the hepatectomy and MELD score (Figure).



P0387

IDENTIFICATION OF OPTIMAL CUT-OFFS IN ALPHA-FETOPROTEIN AND THE "AFP SCORE" TO MAXIMISE THE ACCURACY OF SELECTION OF LIVER TRANSPLANT CANDIDATES WITH HEPATOCELLULAR CARCINOMA

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Background and Aims: Accurate prediction of HCC recurrence is critical in selection of candidates for liver transplantation (LT) to maintain acceptable outcomes. Current selection criteria include tumour size and number, serum alphafetoprotein (AFP) concentration, or combinations such as the AFP Score (Duvoux et al, Gastroenterology 2012). The optimal cut-off for AFP has not been defined and multiple, frequently arbitrary, cut-offs have been used. The cut-off for the AFP Score was defined at ≤2 in the original report but this has not been explored in other populations. The aim was to determine optimal cut-offs for AFP and the AFP Score in UK transplant recipients.
Methods: Patients from 3 UK centres, transplanted between 1996 and 2011 were included. Tumour size and number on pre-LT imaging, and AFP from the time of listing were analysed. Optimal cut-offs for the prediction of HCC recurrence by AFP and the AFP Score were determined using receiver operator characteristic (ROC) curve analyses in a derivation cohort of 209 patients from one centre. Cut-offs were then validated in a cohort of 294 patients from two independent centres.
Results: The characteristics of the derivation cohort were: median age 58.5 years, 82% male, 44% HCV infection, and 85% within the Milan criteria on imaging pre-LT. The area under the ROC curve was not different for AFP alone (0.74; 95% CI 0.62–0.85) and the AFP Score (0.74; 95% CI 0.64–0.84). Using traditional methods to select cut-offs the identified specificity values were between 0.71 and 0.76. The use of cut-offs based on these methods excluded 10–25% of patients with potentially favourable outcomes post-LT. Cut-offs were therefore identified by setting specificity at 0.9 to maximise opportunities for LT. Using this method the cut-off for AFP was found to be 497 (subsequently rounded to 500), and for AFP Score to be ≤3. Implementing these cut-offs in the validation cohort allowed identification of patients with low vs. high risk of recurrence after LT: AFP, 5-year recurrence risk 13.0 vs. 34.8% ($p<0.05$); AFP Score 5-year risk 12.8 vs. 44.1% ($p<0.001$).
Conclusions: Using traditional methods to identify cut-offs in selection of transplant candidates with HCC identifies cut-offs that are insufficiently specific. This disadvantages many patients who would otherwise benefit from transplantation. In individuals largely within the Milan criteria a cut-off of AFP 500 or an AFP Score of ≤3 increases the accuracy of candidate selection whilst still permitting transplantation in the majority.

P0388

SURGICAL RESECTION VERSUS RADIOFREQUENCY ABLATION FOR SINGLE HEPATOCELLULAR CARCINOMA ≤2 cm IN A PROPENSITY SCORE MODEL

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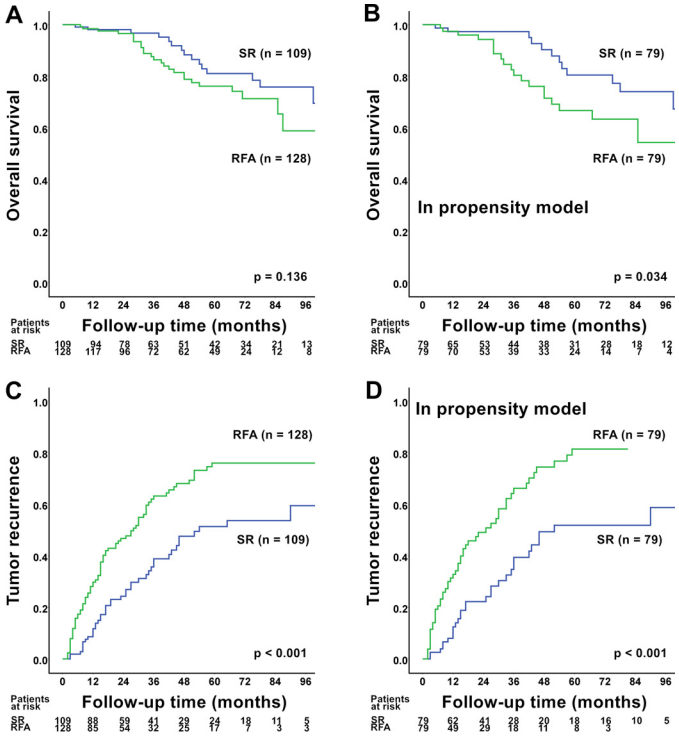
Background and Aims: The long-term therapeutic efficacy between surgical resection (SR) and radiofrequency ablation (RFA) for single hepatocellular carcinoma (HCC) ≤2 cm remains undetermined.
Methods: Between 2002 and 2013, 237 (SR in 109 and RFA in 128) patients with Barcelona Clínic Liver Cancer (BCLC) very early stage HCC were enrolled. Their overall survival (OS) and recurrence-free survival (RFS) were compared. Propensity score matching analysis identified 79 matched-pairs of patients to compare the outcomes.
Results: At baseline, SR patients were younger and had larger tumor (both $p < 0.05$). The 5-year OS rates were 81% for SR and 76% for RFA group ($p = 0.136$, Figure A), and 5-year RFS rates were 49% *versus* 24% for SR and RFA, respectively ($p < 0.001$, Figure C). In the propensity model, the baseline variables were well-balanced between two treatment groups. SR was significantly associated with better OS and RFS compared with RFA; the 5-year OS rates were 80% *versus* 66% ($p = 0.034$, Figure B), and 5-year RFS rates were 48% *versus* 18% ($p < 0.001$, Figure D) for SR and RFA group, respectively. The Cox proportional hazards model identified RFA as an independent predictor for mortality and tumor recurrence in the propensity model (hazard ratio: 2.120 and 2.421, respectively; both $p < 0.05$). Patients with recurrent HCC had inferior prognosis compared with patients without recurrence ($p = 0.001$). However, the survival after recurrence was similar between patients initially treated with SR or RFA ($p = 0.415$).



Conclusions: SR provides better long-term OS and RFS compared to RFA in patients with BCLC very early stage HCC. SR should be considered as the first-line treatment for these patients.

P0389

CAN WE PREDICT MICROVASCULAR INVASION IN HCC ON FDG PET-CT?

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Background and Aims: The purpose of this study is to correlate clinicopathologic and PET-CT parameters with the presence of microvascular invasion (MVI) at histopathologic examination (HPE) in patients with hepatocellular carcinoma (HCC) who underwent liver transplantation.
Methods: We assessed 224 patients with HCC undergoing liver transplantion and a pre-transplant PET-CT. Three physicians (two nuclear physicians and one radiologist) analyzed the following tumor parameters in consensus: size, multi-focality, pattern of uptake, quantitative FDG uptake (SUV), pattern of enhancement and distance to closest vessel. The size and number of lesions, tumor differentiation and the presence or absence of microvascular invasion were determined at HPE and these findings were analysed vis-a-vis to the imaging parameters on PET-CT.
Results: None of the clinical parameters was predictive of MVI; however on uni-variate analysis, MVI was significantly associated with multi-focality, uptake pattern and distance to the closest vessel on FDG PET-CT. By applying multiple logistic regression analysis, uptake pattern (heterogeneous and peripheral FDG uptake) was found to be the only independent risk factor for MVI.
Conclusions: Heterogeneous and peripheral FDG uptake on PET-CT was the only parameter that correlated significantly with MVI.

P0390

BASELINE BILIRUBIN AND NOT CHILD–PUGH A OR B7 PREDICTS SURVIVAL OF BCLC B PATIENTS TREATED WITH SORAFENIB. THE CLINICAL APPROACH TO THE STRATIFICATION DEBATE

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Background and Aims: BCLC-B stage includes multifocal hepatocellular carcinoma (HCC) without extrahepatic disease, Child–Pugh score (CP) A–B and performance status (PS) 0. In some cases the recommended treatment for this stage (TACE) is discarded for unfeasibility or failure. Thus, a relevant percentage of patients is considered for sorafenib [treatment stage migration (TSM) or untreatable progression (UP) concepts]. Some authors have proposed to stratify the BCLC-B stage according to CP points but this dismisses the fact that CP >7 patients require transplant consideration if the HCC burden does not exceed selection criteria. If this is the case, the hepatic impairment prevents treatment benefit. The aim of this study is to identify predictors of survival (OS) in properly selected BCLC-B patients treated with sorafenib.
Methods: We prospectively included BCLC-B patients (PS 0, no vascular invasion/extrahepatic spread), CPA/B7 without encephalopathy/clinical ascites at sorafenib start (800 mg/day). Follow-up was performed with 4 week-clinical/laboratory monitoring and radiological control at 4 wks and then every 8 wks. Treatment continued until symptomatic progression, intolerance or entry in 2nd line trials.
Results: Between October 2007 and March 2014, 99 BCLC-B patients began sorafenib (86% males, 58% HCV, 81% CPA). After a median follow-up and treatment duration of 12.7 and 8.3 months (P33 5.4;

P66 11.6), the median OS was 14.8 months (14 if censored at 2nd line). OS did not differ according to CP (CP A: 13.9 months; CP B: 12.7). The uni–multivariate analysis of CP parameters identified baseline bilirubin (TB) as the only independent OS predictor. OS was significantly longer in patients with normal TB (TB <1.2 mg/dl) vs. those with elevated TB (16.3 months vs. 12.3 months). The predictive role of TB is kept at the same HR and 95% CI irrespective of the baseline variables included in the model: CP parameters and previous decompensation; CP parameters and ascites controlled by diet +/− diuretics; CP parameters and BCLC-B naive/BCLC-B due to TSM or UP baseline characteristics + CP parameters + previous decompensation + ascites controlled by diet +/− diuretics [TB HR = 1.64, 95% CI (1.147–2.357) in all analysis].
Conclusions: Stratification of patients into Child–Pugh A vs. B7 does not predict OS in this population and its use does not provide useful information. Contrarily, baseline TB should be used for prediction and prognostic stratification of BCLC-B patients treated with sorafenib.

P0391
PRIOR HISTORY OF ARTERIAL HYPERTENSION IS NOT A CONTRAINDICATION TO SORAFENIB AND IS ASSOCIATED TO A BETTER SURVIVAL IN PATIENTS WITH ADVANCED HCC

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Background and Aims: Sorafenib is a multikinase inhibitor with antiangiogenic/antiproliferative effect. It presents a specific pattern of adverse reactions: dermatological reactions, hypertension (HT), hypophosphatemia and diarrhea. It is controversial if this drug is tolerable in patients (pts) with history of HT or if such comorbidity may influence overall survival (OS). This prospective study analyzes if prior existence of HT is associated with worse tolerance and lower efficacy of sorafenib and to evaluate if this comorbidty may affect OS.
Methods: We included patients with HCC, Child–Pugh (CP) A or B7 points, without encephalopathy or clinical ascites, with controlled arterial pressure (AP) and without active cardiovascular disease in an intensive program of cardiovascular evaluation. Clinical and laboratory examination was done every 4 weeks and CT-scan before starting the treatment, after 4 weeks and then every 8 weeks. All but 11 pts started at full dose (800 mg/day). Dose adjustments were applied according to clinical tolerance and therapy was maintained until symptomatic progression, intolerance or inclusion in 2nd line trials.
Results: Between October 2007 and March 2014, 208 pts started sorafenib and 105 were included: 93% male, 53.5% HCV, 54% BCLC-C, 89% CP-A, 93% PS0; 49% of the pts had prior history of HT and most were under treatment (47/52 pts). Median systolic AP was 128 mmHg (90–169) and diastolic AP was 78 (50–96), 26% of the pts were diabetics. Median follow up, treatment duration and OS were 11.2 months (mo), 6.1 mo and 12.7 mo, respectively. Pts with HT did not require more dose modifications or treatment interruptions, than pts without HT. In multivariate analysis, none of the analyzed factors (HT, treatment of HT, basal values of AP, diabetes) was predictor of OS. However, if stratifying pts according to history of HT and values of AP as CTCAE 4.03, those with normal values showed longer OS [17.6 vs 9.42 months (p = 0.025); HR: 2.955 (95% CI 1.103–7.917) p = 0.031]. Similarly, pts with optimal or normal values of AP (≤129/84 mmHg; n = 47) according to the European Society of Cardiology and with prior history of HT, showed better survival [17.6 vs 10.4 months (p = 0.037), HR: 2.296 (95% CI 1.033–5.106) p = 0.041].
Conclusions: History of HT is not a contraindication to sorafenib in pts with advanced HCC if AP is adequately controlled. Treatment duration and tolerability are not affected by HT and OS may be even longer in pts with HT.

P0392
TRANSARTERIAL CHEMOEMBOLISATION IN PATIENTS WITH METASTATIC HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma (HCC) is often diagnosed in an advanced stage and therefore treatment is limited due to impaired liver function and the lack of highly effective systemic treatment possibilities. Sorafenib is the treatment of choice in advanced metastatic HCC. Several studies have shown that treatment with transarterial chemoembolisation (TACE) in metastatic HCC can improve overall survival (OS) in these patients. It was therefore the aim of our study to investigate efficacy of TACE and and its impact on OS in patients with metastatic HCC.
Methods: 215 Patients with metastatic HCC who were treated at our Liver Center between 2003 and 2014 were included in the analysis. Medical records, laboratory parameters and imaging studies were analyzed. Treatment of metastatic HCC, efficacy and OS were assessed.
Results: The study population compromised 215 patients with metastatic HCC (185 [86%] male, median age 69 years). The main cause of liver disease was alcohol (35.8% of patients). 85% of our patients were classified as BCLC C whereas the remaining 15% were in BCLC D. Most common sites of metastases were the lung (48.8%) and extrahepatic lymph nodes (46.5%). Sorafenib treatment was performed in 71 of 215 patients (33%), TACE in 65 of 215 patients (47.1%) and a combination of both in 23 patients. Patients treated with TACE were all in BCLC C. In patients without multiple metastases treatment with TACE led to a significantly better OS compared to no TACE treatment (12 vs. 4 months, p < 0.001). Multivariate Cox regression analyses revealed TACE (HR 0.62; 95% CI:0.39–0.97, p = 0.037), multifocal intrahepatic HCC (HR 2.21; 95% CI:1.27–3.84, p = 0.005) and MELD score (HR 1.10; 95% CI:1.03–1.18, p = 0.005) as significant independent prognostic factors in these patients.
Conclusions: In advanced, metastatic HCC treatment of intrahepatic tumor using TACE may be associated with improved survival highlighting the prognostic importance of treating intrahepatic HCC even in patients with metastatic disease.

Molecular and cellular biology: a. Cell cycle control/Apoptosis

P0393
SELF-ASSEMBLED LIVER ORGANOIDS RECAPITULATE HEPATO-BILIARY ORGANOGENESIS IN VITRO

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Background and Aims: Bioengineering of a fully functional tissue requires precise recapitulation of normal tissue development.

Specifically for the liver, one may use bipotent human liver progenitor cells (hFLCs) capable of differentiation into hepatocytes and cholangiocytes. The goal of the current study was to develop a system that would efficiently recapitulate embryonic development of hepatic parenchymal tissue and bile ducts, using decellularized liver extracellular matrix (ECM) as scaffolds.

Methods: hFLCs were seeded on decellularized liver ECM discs (300 μm thickness, 8 mm diameter) and were cultured for up to 3 weeks in presence of hepatic differentiation medium. Immunofluorescence microscopy was used to determine the extent of progenitor cell differentiation into hepatocytes and cholangiocytes. Urea, albumin and drug metabolism were quantified as paramaters of liver function.

Results: hFLCs seeded on acellular liver ECM discs differentiated into hepatocytes and cholangiocytes. The cells showed predominant albumin expression along with loss of α-feto protein (AFP) expression at 3 weeks (Fig. 1D,E). The cells also expressed other mature hepatocyte markers like HNF-4α, α-1-antitrypsin and cytochrome P450 1A2, 2A and 3A (Fig. 1B–E). The cells in the ductular structures expressed bile duct specific markers like CK19, SOX9, EpCAM, ASBT, β-catenin and the presence of apical primary cilia (stained with α-acetylated tubulin), thus demonstrating differentiation towards cholangiocyte lineage along with maintaining apico-basal polarity (Fig. 1B–E). Urea and albumin secretion was higher in the liver disc organoids compared to control hFLCs cultured in petri dishes. Several metabolites of the drugs diazepam and 7-ethoxycoumarin were also detected by LC-MS/MS, showing broad cytochrome P450 activity in these organoids.

Conclusions: Our results demonstrate the efficient generation of bioengineered human liver tissue with hFLC that recapitulates stepwise development of hepatocyte and bile duct formation (Fig. 1A). Altogether, this study demonstrates the potential of this technology to study and mimic human liver development. These models provide novel approaches for liver bioengineering, drug discovery and toxicology, and ultimately for the treatment of liver disease.

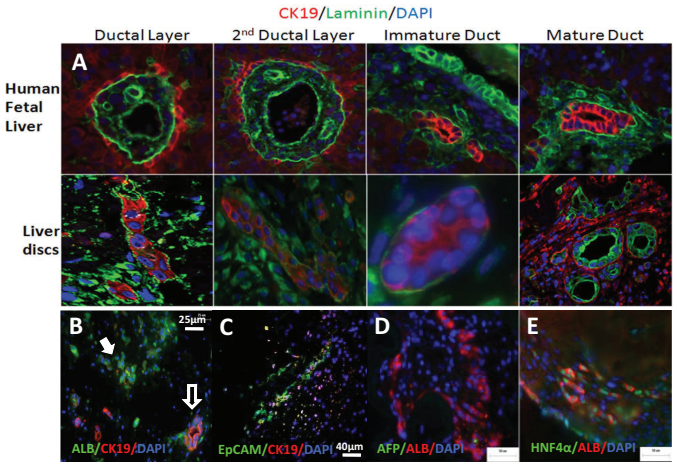

Figure 1.

P0394

ACUTE BLEEDING AND STORED RBC TRANSFUSION ATTENUATES LIVER REGENERATION FOLLOWING PARTIAL HEPATECTOMY

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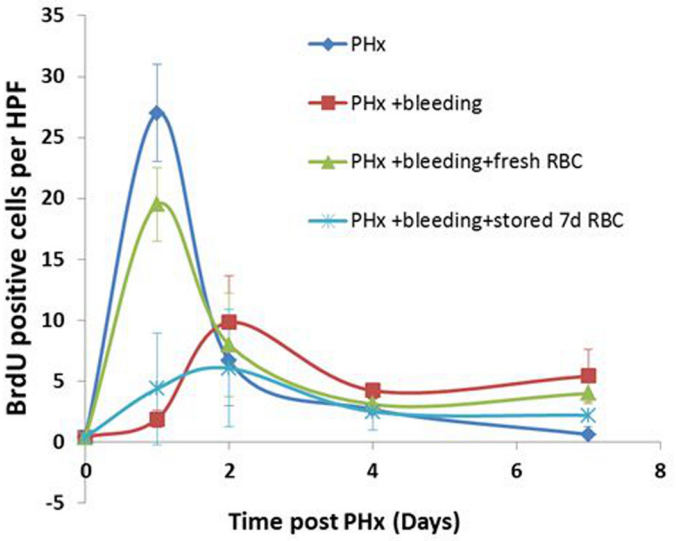
Background and Aims: Liver regeneration, a process that rapidly compensates for the acute loss of liver parenchyma, plays a critical role in patients requiring large liver resections or living-donor liver transplantation. These operations are frequently associated with massive blood loss with subsequent red blood cell (RBC) transfusion. Avoiding further liver injury in the remaining liver parenchyma and inhibition of liver regeneration is of profound clinical importance. While the liver is one of the targets for injury in low flow states associated with hemorrhage, concerns have recently been raised about the safety and efficacy of RBC transfusion. In a rat model of partial hepatectomy (PHx), our aims were to study the impact of blood loss on liver regeneration, and to evaluate whether RBC transfusion modifies the liver regeneration processes.

Methods: Liver regeneration was assessed in a rat model of 50% PHx. Rats were randomized into 4 experimental groups: (I) Control hepatectomy (PHx; n = 24); (II) bleeding (3 ml) and PHx (n = 24); (III) bleeding (3 ml) and PHx followed with fresh RBC resuscitation (n = 24); (IV) bleeding (3 ml) and PHx followed with "aged" RBC resuscitation (n = 24; RBC stored for 7 days). Rats were sacrificed at 1, 2, 4 and 7 days after PHx, bleeding and resuscitation (6/time point) for histological evaluation, for serum liver enzymes (ALT and AST) and for cytokines levels (IL-6 and TNFa). Histological samples were immunostained with BrdU for assessment of hepatocyte proliferation.

Results: Acute bleeding interfered with the regenerative process of the liver following PHx. In rats subjected to 50% PHx and bleeding, liver regeneration was delayed and hepatocyte proliferation significantly reduced when compared to rats that had only PHx (figure). Resuscitation with fresh RBC but not with "aged" RBC, restored liver regeneration kinetics (figure). The delay in liver regeneration due to acute bleeding was associated with increased liver injury, as assessed by elevated ALT and AST levels. IL-6 is critical for initiation of liver regeneration process. Plasma IL-6 levels were increased at 24 hours post PHx and with RBC transfusion, however, were significantly reduced with PHx and bleeding.

Conclusions: Avoiding blood loss and transfusion of stored RBC in patients undergoing PHx is of great importance to ensure the regenerative period. Resuscitation with fresh RBC restores liver regeneration capacity.



P0395

MIR-22-TARGETED CYCLIN A EXPRESSION IN GI CANCER CELLS IS REGULATED BY BILE ACID RECEPTOR

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Background and Aims: Due to the significant tumor suppressive role of microRNA-22 (miR-22), it is important to understand the mechanism by which miR-22 is expressed and regulated. Identification of miR-22 downstream targets would help us to understand its tumor suppressive effect as well. Farnesoid X receptor (FXR) is abundantly expressed in the liver and intestines and plays a pivotal role in maintaining the health of the gastrointestinal tract. As a transcriptional factor, FXR can exert its biological effects by regulating microRNAs.
Methods: Using standard molecular biology approaches, the current study was designed to understand the regulation of miR-22 by FXR and identify additional downstream miR-22 targets in liver and colon cell lines.
Results: The results showed miR-22 was transcriptionally regulated by FXR through direct binding to an invert repeat-1 (IR-1) motif located at 1012 to 1025 bp upstream from miR-22. Among the studied primary and secondary bile acids, chenodeoxycholic acid (CDCA), which has the highest binding affinity to FXR, induced miR-22 level in both Huh7 liver and HCT116 colon cells in a dose-dependent manner. In addition, cyclin A2 (CCNA2) was identified as a miR-22 novel target in liver and colon cancer cells. The sequence of miR-22, which is conserved in mice, rats, humans, and other mammalians, aligns with the sequence of 3′ UTR of CCNA2. CDCA treatment and miR-22 mimic reduced the expression of CCNA2 in Huh7 and HCT116 cells. In mice, reduction of miR-22 is accompanied by the induction of hepatic and ileal CCNA2 protein levels in 3 month old FXR knockout (KO) mice. In human specimens, the expression levels of miR-22 and CCNA2 are inversely correlated in liver and colon cancers.
Conclusions: Taken together, our data showed that FXR-induced miR-22 in suppression of CCNA2 is a novel pathway for FXR to exert its protective effect in the gastrointestinal tract.

P0396

EMRICASAN, A POTENT PAN CASPASE INHIBITOR, RAPIDLY REDUCES CASPASE ACTIVITY AND BIOMARKERS OF APOPTOSIS IN PATIENTS WITH HEPATIC IMPAIRMENT BUT NOT IN HEALTHY VOLUNTEERS: IMPLICATIONS FOR SAFETY, SELECTIVITY AND MECHANISM OF ACTION

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Background and Aims: Emricasan, (formerly IDN-6556) is a potent, orally active caspase inhibitor currently in five on-going Phase 2 clinical trials, in patients with various etiologies and severity of liver disease. Caspases are enzymes that are critically involved in apoptosis and the maturation of the pro-inflammatory cytokines, IL-1and IL-18. Apoptosis and caspase activity are elevated in many liver diseases and are closely associated with disease severity and progression. We have studied the effect of emricasan on caspase activity and markers of apoptosis in subjects with hepatic impairment, severe renal impairment and matched healthy volunteers.
Methods: Emricasan was administered as a single 50 mg oral dose to subjects with mild (n = 12), moderate (n = 8) or severe (n = 8), hepatic impairment and 8 matched healthy controls. In a second study, emricasan was administered as a single 50 mg dose to 8 subjects with severe renal impairment and 8 matched healthy controls. In both studies, serial blood samples were collected over a 48 hour period and analyzed for markers of apoptosis, cell death and caspase enzymatic activity using, M30 Apoptosense, M65 and caspase Glo 3/7 assays, respectively.
Results: All 28 subjects with hepatic impairment experienced rapid and significant reductions, ($p < 0.05$), relative to Baseline for all of these markers of mechanistic activity and pharmacodynamic response following a single 50 mg oral dose of emricasan. In subjects with severe renal impairment, caspase activity and M65 were elevated relative to controls and emricasan had no effect on these markers. Emricasan also had no effect on caspase activity or markers of apoptosis the cohorts of healthy volunteers.
Conclusions: Emricasan did not reduce levels of caspase activity in healthy volunteers or in subjects with severe renal impairment following a single 50 mg oral dose. However, emricasan efficiently reduced caspase activity and markers of apoptosis and general cell death in all subjects with hepatic impairment. Combined, these observations suggest that reduction in systemic caspase activity result from reduced efflux of active caspase from diseased organs rather than inhibition of caspase activity in the systemic circulation. These observations provide new and potentially fundamental insight into the cellular mechanisms of how emricasan affects apoptosis. They also suggest that emricasan is uniquely suited to treat liver diseases where excessive caspase production is implicated.

P0397

Nrf2 MUTATIONS ARE AN EARLY AND FREQUENT EVENT IN THE DEVELOPMENT OF RAT HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma (HCC) is a multistage process, but the nature of the molecular changes associated to the different steps, particularly the very early ones, is unknown. The Nrf2–Keap1 pathway is now recognized as a major cellular defense mechanism against oxidative and electrophilic stresses; however, its exact role in cancer development is unclear.
Methods: To unveil the role of Nrf2 in HCC, we used the Resistant-Hepatocyte (R-H) rat model, which identifies distinct lesions (preneoplastic foci, dysplastic nodules, early and advanced HCCs) at well-defined timings. Identification of *Nrf2/Keap1* mutations and the expression of target genes of this pathway were performed by Sanger analysis and qRT-PCR. Immunohistochemistry was used to detect the activation of the pathway in the pre- and neoplastic lesions.
Results: Mutations of *Nrf2* are very frequent in early preneoplastic lesions (70%). All the mutations were missense, involved the Nrf2-Keap1- binding regions and were associated with a strong increase of expression of the *Nrf2*-target genes (*Nqo1, Gclc, Gst4)*, in both pre- and neoplastic lesions. HCCs arising 10 and 14 months later also showed a high percentage of *Nrf2/Keap1* mutations (70 and 56%, respectively), suggesting that mutation of these genes is critical for HCC development. *Nrf2* silencing inhibited the ability of rat tumorigenic cells to grow in soft agar and to develop HCC when injected into syngeneic rats.
Conclusions: This study demonstrates that *Nrf2* mutation is a very early and frequent event, suggesting that dysregulation of the Nrf2-Keap1 pathway is likely essential for the clonal expansion of preneoplastic hepatocytes to HCC.

P0398

CHANGE OF FIBROSIS PATTERN AFTER AUTOLOGOUS BONE MARROW CELL INFUSION IN PATIENTS WITH ADVANCED LIVER CIRRHOSIS

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Background and Aims: Although we previously published positive clinical results of human clinical trial of autologous bone marrow cell infusion (ABMI) therapy for advanced liver cirrhosis (LC) (Kim JK et al. Cell Transpl 2010), change of fibrosis stage could not be shown. Because the patients had thick advanced fibrotic band, small change could not be evaluated by current staging system. Recently, enhanced detection and quantification of collagen fibers are possible using the nonlinear optical microscopy. The aim of this study is to show the change of fibrosis after ABMI in patients with advanced LC.
Methods: Patients with Child–Pugh B or C LC and no viable hepatocellular carcinoma (HCC) underwent ABMI. Autologous BMCs were harvested and infused into peripheral vein after RBC depletion and mononuclear cell concentration. Patients were followed up every month during first 6 months of study period after the ABMI. Liver biopsy was performed before ABMI, and 1, 3, and 6 months after ABMI, if the patient agreed. Repeated biopsy samples were imaged by Genesis™ system (Histoindex, Singapore), a second harmonic generation and two-photon excitation fluorescence technology-based commercial devise. Collagen features were divided into portal, septal or fibrillar collagen as previous report (Xu et al. J Hepatol 2014).
Results: Twenty patients were screened and 19 patients (M:F=9:10) were enrolled. Mean age was 52 year-old. Repeated biopsy samples were available in 8 patients for this study. Seven patients had B-viral LC and one had alcoholic LC. Only at 3 months after ABMI, aggregated collagen, string area, number of crosslinks, portal collagen percentage, portal aggregated collagen percentage, portal string area, portal and aggregated string area, portal and aggregated number of thin strings, portal number of crosslinks, septal number of thin strings, septal string area, septal and aggregated string area, fibrillar number of strings, fibrillar string area, fibrillar and aggregated string area were significantly decreased compared to baseline ($p < 0.05$, respectively).
Conclusions: ABMI improved not only hepatic function but also collagen deposition. However, significant collagen decrease was noted only at 3 months after ABMI.

P0399

Notch1 IS A MASTER REGULATOR OF THE SENESCENCE SECRETOME THROUGH REPRESSION OF CEBPβ

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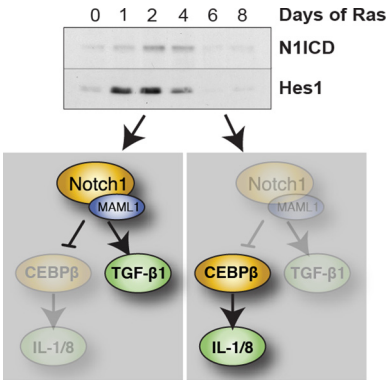
Background and Aims: Oncogene-induced senescence (OIS) is an intrinsic tumour suppressor mechanism, but its impact on tumorigenesis is largely dependent on the nature of SASP, the senescence-associated secretory phenotype. Major components of the SASP include TGFβ1 and pro-inflammatory cytokines, such as IL1, IL6, and IL8, that have pleiotropic context-dependent effects.
Methods: We utilised the well validated ER:Ras^{G12V} IMR90 HDF in vitro model which undergo Ras-induced senescence (RIS) with 4OHT. Genetic manipulation was achieved through retroviral gene transfer; transcriptional profiling by mRNA-seq; validation through qPCR and immunoblotting.
Results: Previously, we have shown that Notch1, a highly conserved receptor is up-regulated in RIS. In contrast to the up-regulation of Notch1, downstream signaling is dynamically regulated: the cleaved, active intracellular domain of Notch1 (N1ICD) and Notch-target genes were transiently up-regulated at an early phase of RIS, but down-regulated at full senescence. The dynamic expression pattern of N1ICD and TGF-β1 expression were nearly identical, and inversely correlated with the cytokines, IL1 and IL8. Inhibition of Notch1 signaling, through expression of a dominant-negative form of the Notch1 binding partner MAML1, led to a reduction in TGF-β1, but increased IL1 and IL8 expression during RIS. In addition, ectopic restoration of N1ICD in established RIS cells drove reciprocal secretome changes with reduced IL1 and IL8 and increased TGF-β1, suggesting Notch1 signaling plays a critical role in secretome switching.
It has been shown that the SASP in RIS is regulated by two major transcription factors, NFkB and CEBPβ. Strikingly, over-expression of N1ICD strongly down-regulated CEBPβ, but not NFkB, in fully established RIS cells. Further, expression of ectopic CEBPβ in N1ICD-expressing cells partially restored levels of IL6/8. These data indicate that Notch1 represses pro-inflammatory cytokines by down-regulating CEBPβ. Finally, Notch1 up-regulation in RIS was confirmed in several mouse models: the murine Kras-driven pancreatic intraepithelial neoplasia and Nras-driven hepatocyte senescence models.
Conclusions: We propose that the transition to OIS is correlated with a switch from Notch1-driven TGFβ1-rich secretome to a CEBPβ-driven IL1/8 rich secretome, and that dynamic Notch1 signaling modulates senescence and its long-term fate strictly through a non-cell-autonomous fashion.



P0400

MICRORNA SIGNATURE OF EARLY-STAGE HEPATOCELLULAR CARCINOMA ARISING IN HCV-RELATED CIRRHOSIS

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Background and Aims: Systems currently used to stage hepatocellular carcinomas (HCCs) perform poorly in prognostic settings. The availability of tumor biomarkers that reliably correlate with disease outcome would facilitate treatment and follow-up strategy planning. To identify such markers, we analyzed the microRNA profiles of HCCs and the cirrhotic tissue in which they develop.
Methods: Ten patients with HCV-related cirrhosis underwent resective surgery for solitary, early-stage HCCs. In each case, we

collected one sample of tumor and two samples of tumor-free cirrhotic tissue (near and at a distance from the tumor) and used them to construct 30 small RNA libraries for next-generation sequencing. The reads that emerged were clustered and aligned against the MiRBase with CLC Genomic Workbench software.
Results: A total of 2,329 mature microRNAs were found. Annotated microRNAs with a mean read number per sample of >20 underwent parametric and randomized statistical analyses. Those associated with *P values* of ≤0.01 in both analyses were considered differentially expressed between tissue types. Of the 617 microRNAs subjected to statistical analysis, 35 exhibited expression levels in HCC that were significantly different from those found in one or both tumor-free cirrhotic tissues; two of the 35 were also differentially expressed in the two types of cirrhotic tissue. Eighteen of the 35 had never been reported as dysregulated in HCC.
Conclusions: We identified an HCC-signature set of 35 microRNAs, which distinguishes HCCs from background cirrhotic tissues. It should provide a good starting point for subsequent research aimed at identifying biomarkers correlating with HCC outcome.

P0401
THE SERINE PROTEASE FSAP (FACTOR VII ACTIVATING PROTEASE) MAINTAINS THE DIFFERENTIATED STATE OF MOUSE HEPATOCYTES
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Background and Aims: FSAP, encoded by the *Habp2* gene, is a serine protease secreted by hepatocytes as an inactive zymogen that circulates in the blood. We have recently demonstrated a novel anti-fibrotic role for FSAP in the liver. The aim of this study was to analyze the effect of FSAP on hepatocyte differentiation and proliferation.
Methods: Primary hepatocytes isolated from BALB/c wild type (WT) or FSAP-deficient mice and the mouse hepatocyte cell line AML-12 were used as *in vitro* models. The use of knockout hepatocytes was further supported by the use of siRNA-mediated knock-down of the gene for FSAP and the addition of exogenous FSAP protein. Transcript levels were analyzed by qRT-PCR, protein levels by Western blotting and DNA synthesis by BrdU incorporation.
Results: Primary hepatocytes and AML-12 cells lacking expression of endogenous FSAP show a reduction in HNF4α (Hepatocyte Nuclear Factor 4α) and an increase in AFP (alphafetoprotein) transcript levels compared to WT cells. Besides, Transforming Growth Factor beta-1 (TGFβ) almost completely abolishes HNF4α mRNA levels in FSAP-depleted cells, while the reduction in WT cells is around 50%. Basal reduction on HNF4α mRNA is not due to an Epithelial-Mesenchymal Transition (EMT): silencing of the gene for FSAP does not down-regulate E-cadherin protein levels nor up-regulates mRNA levels of the EMT-inducing transcription factors (TFs) SNAI1, ZEB1 and ZEB2. Moreover, FSAP depletion does not impair the TGFβ-induced up-regulation of these TFs.
Preliminary results show that exogenous FSAP induce a 30% increase in basal BrdU incorporation in hepatocytes. Exogenous FSAP can overcome the anti-proliferative effect of TGFβ in FSAP-deficient hepatocytes, but not in WT cells, suggesting that lack of endogenous FSAP decreases the sensitivity to TGFβ inhibitory effects.
Conclusions: We have for the first time described a novel role for FSAP in hepatocyte differentiation. It contributes to mantain HNF4α expression and AFP repression but does not affect EMT. This, together with its potential role on proliferation, provides a mechanistic insight into the effect of FSAP on hepatocytes that is relevant for hepatocellular carcinoma.

P0402
COMBINATION THERAPY FOR HEPATOCELLULAR CARCINOMA: A SYSTEMS BIOLOGY PERSPECTIVE ON THE SYNERGISTIC ANTITUMOR ACTIVITY OF SORAFENIB WITH PI3K/AKT PATHWAY INHIBITORS
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Background and Aims: Sorafenib, a multi-kinase inhibitor with anti-angiogenic functions, is the only FDA-approved molecular-targeted agent for the treatment of patients with advanced Hepatocellular carcinoma (HCC). Yet, Sorafenib shows limited overall survival benefit associated with resistance and tumor recurrence. Current mono-target- or single pathway-centric drug designs are not sufficient for effective therapy of advanced HCC. Since Sorafenib targets angiogenic VEGFR and PDGFR kinases and RAF/MEK/ERK signaling, the primary mechanism of resistance to Sorafenib and tumor recurrence in HCC patients emerges to be the compensatory PI3K/AKT signaling. In this study, we analyzed the synergistic effects of Sorafenib and PI3K/AKT inhibitors on HCC cell growth, determined possible mechanisms underlying synergistic mechanism of action by transcriptome analysis, and further showed that combination therapy leads to tumor regression in HCC xenografts *in vivo*.
Methods: Cytotoxic activities of the PI3K/AKT pathway small molecule inhibitors were shown by SRB and RT-CES assays in HCC cell lines having normal or hyperactive AKT kinase. Apoptotic cell death and suppression of cell cycle progression were shown by flow cytometry, immunofluorescence and western blots experiments. Transcriptome profiling of inhibitor treated cells were performed with RNA-sequencing. Therapeutic efficacy of combination therapy was shown *in vivo* in athymic mice bearing HCC xenografts.
Results: We showed the cytotoxic activities of isoform specific PI3K inhibitors and AKT inhibitors (Akti-1,2 and Akti-2) alone and in combination with Sorafenib *in vitro*. We revealed the molecular mechanisms of action of single agent and combination therapies using transcriptome profiling. We determined the predominant role of PI3K isoform p110α in PTEN-deficient HCC cells. We showed that combining Sorafenib with PI3K/AKT inhibitors enhances anti-tumor efficacy of Sorafenib and results in tumor regression *in vivo*.
Conclusions: Our results provide *in vitro* and *in vivo* experimental evidence of the therapeutic potential of combination therapies with Sorafenib and PI3K/Akt inhibitors for the treatment of advanced HCC.

P0403
STEATOTIC LIVER AS A SOURCE OF HEPATIC PROGENITOR CELLS WITH THERAPEUTIC POTENTIAL
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Background and Aims: Ductular reactions (DRs) are observed in chronic liver injuries. In mice, ductular reactions have been studied extensively in models such as Choline-Deficient Ethionine (CDE) supplemented diet and 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet. On contrary, DRs in models of fatty liver disease is less studied. We aim to investigate the DR in fatty liver disease and the plausibility to isolate Hepatic Progenitor Cells (HPCs) from fatty liver to use as an alternative to whole organ transplant.
Methods: C57BL6 mice were supplemented with High Fat Diet, Choline Deficient (CD) diet, Methionine-Choline Deficient (MCD)

diet to activate DRs. HPCs were isolated by Fluorescence Activated Cell Sorting (FACS) based on previously described HPC markers (CD45-/CD31-/Ter119-/EpCAM+/CD24+). HPCs were cultured at clonal density either in a conventional two dimensional system or a three dimensional manner to form liver organoids. *In vitro* expanded HPCs were transplanted into recipient mice with liver injury via intrasplenic injection. Recipient livers were analysed for engraftment and liver repopulating capacity of HPCs.

Results: Mice fed with MCD diet have the most robust DR among the models of steatosis we tested. The degree of DR is even comparable to the widely used CDE diet (10 fold higher than healthy control). Isolated HPCs can be expanded *in vitro* in both 2-dimensional and 3-dimensional manner. Expanded HPCs maintain the expression of HPC markers, CK19, Sox9, and Osteopontin after long term culture. Furthermore, expanded cells are phenotypically stable with normal chromosome number. Expanded HPCs from MCD liver express the same markers as HPCs isolated from CDE treated liver, with similar expression of HPC marker EpCAM, CD24, CD133, LGR5. Expanded HPCs can differentiate along the hepatocyte lineage and are capable of synthesising albumin and store glycogen *in vitro*. Transplanted HPCs engraft and repopulate the liver parenchyma in a large scale after liver injury.

Conclusions: We found that mice supplemented with Methionine-Choline Deficient (MCD) diet, a model of steatohepatitis have robust DRs. HPCs isolated from MCD treated liver can be expanded in vitro and repopulate the liver after transplantation. This proves the potential of using HPCs isolated from fatty liver that are rejected for transplant for cell therapy in the future.

P0404

CYCLIN E1 EXPRESSION LEVEL DETERMINE THE ANTI-PROLIFERATIVE RESPONSE OF THE PHARMACOLOGICAL Cdk2 INHIBITOR ROSCOVITINE IN HEPATOMA CELLS AND LIVER

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Background and Aims: The cyclin-dependent kinase inhibitor Roscovitine (Rosc) has been regarded as a promising anti-cancer agent. In the present study we investigated the anti-proliferative effects of Rosc in hepatoma cells and liver in order to test if Rosc could be a therapeutic option for the treatment of HCC.

Methods: The properties of Rosc were analyzed in murine hepatoma cells *in vitro* and in mice following partial hepatectomy (PH) *in vivo*. Potential off-target effects of Rosc were investigated in mice with ubiquitous deletion of Cyclin E1 ($CcnE1^{-/-}$), Cyclin E2 ($CcnE2^{-/-}$) or hepatocyte-specific inactivation of Cdk2 ($Cdk2^{\Delta hepa}$), respectively.

Results: *In vitro,* cell cycle activity was dramatically reduced by Rosc in Hepa1–6 hepatoma cells in a dosage-dependent manner. *In vivo*, Rosc treatment significantly diminished DNA-synthesis and completely blocked both Cdk2 and Cdk1 kinase activities in livers of wildtype (WT) mice 48 h after PH. However, 72 h post-surgery, delayed DNA-synthesis, full restoration of Cdk1 kinase activity and slight activation of Cdk2 occurred in Rosc-treated mice. We previously demonstrated that genetic deletion of Cdk2 had no effect on liver regeneration. Intriguingly, Rosc application after PH inhibited DNA-synthesis in WT and $Cdk2^{\Delta hepa}$ livers to the same extend. Thus, the inhibitory effect of Rosc in liver is not mediated *via* Cdk2 but most likely through inhibition of both Cdk2 and Cdk1. Cdk2 activity is controlled by E-Cyclins. To evaluate the role of E-Cyclins for the inhibitory effect of Rosc, $CcnE2^{-/-}$ and $CcnE1^{-/-}$ mice were treated with Rosc and subjected to PH. In $CcnE1^{-/-}$ mice, Rosc treatment resulted in strong cell cycle inhibition after PH. In contrast, Rosc induced only slightly reduced DNA-synthesis in $CcnE2^{-/-}$ mice, which was associated with over-expression of CcnE1. In hepatoma cells with different CyclinE1 expression levels due to conditional gene ablation or adenoviral over-expression we found a strong synergistic cell cycle inhibition by CyclinE1 ablation and Rosc treatment, while adenovirus-mediated CyclinE1 over-expression partially rescued the cell cycle arrest by Rosc.

Conclusions: Rosc may inhibit proliferation of healthy regenerating hepatocytes and immortalized hepatoma cells most likely through blocking of Cdk1 and Cdk2, as initially suggested. The inhibitory effect of Rosc inversely correlates with endogenous levels of CyclinE1 expression. This suggests that only HCC patients with moderate hepatic CyclinE1 expression may benefit from a potential Rosc therapy.

P0405

MOLECULAR MECHANISMS UNDERLYING THE UNPRECEDENTED LIVER REGENERATION INDUCED BY ALPPS SURGERY

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Background and Aims: In many patients with advanced liver cancer a curative resection is not possible with a one-step procedure. A novel surgical approach, ALPPS (Associating Liver Partition with Portal vein for Staged hepatectomy) may be used for unresectable disease due to its ability to induce massive acceleration of liver regeneration. We hypothesize that transection is sufficient to activate plasma proteins necessary for accelerated liver regeneration, and that PVL is necessary to stimulate liver growth.

Using a novel ALPPS mouse model, we aim at studying the molecular mechanisms underlying accelerated liver growth.

Methods: Liver tissue was collected at early time points after Step 1 of ALPPS procedure (30min, 1 h, 4 h, 8 h, 12 h) and analysed by RNA deep sequencing. Plasma samples were collected 30min after ALPPS Step 1 for proteomics analysis by protein enrichment, albumin depletion, and mass spectrometry. Each protein fraction is confirmed for activity by inducing accelerated regeneration upon injection into mice treated with PVL only.

Results: Injection of plasma derived from mice having undergone ALPPS show a significant increase in liver growth compared to plasma from sham-operated mice. Plasma proteins appear to be the major contributors to the accelerated liver growth underlying ALPPS surgery. Transcript analysis shows that differential gene expression occurs 8 hours after the first step of ALPPS. Liver growth is mainly characterized by a hyperplastic reaction reflected as an increased number of hepatocytes entering the cell cycle.

Conclusions: ALPPS is conducive to accelerated liver regeneration through proteins circulating in the plasma as early as 30 minutes after ALPPS Step 1 procedure. Liver growth associated gene expression is observed already within 8 hrs. Identification of plasma proteins stimulating liver growth may have a high therapeutic value in liver disease and associated surgery.

P0406

DELETION OF Cdk1 IN THE LIVER INDUCES CHANGES IN PYRUVATE METABOLISM

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Background and Aims: Conditional loss of Cdk1, a master regulator of cell division, leads to restoration of liver mass

after partial hepatectomy (PHx) without cell division. Lack of hepatic Cdk1 induces changes in the expression of genes involved in mitochondrial metabolism and lipid synthesis, and aerobic glycolysis. Understanding changes in metabolic pathways in the context of cell division in the liver could help to detect novel interactions between cell cycle and metabolism, opening a window to discover enzymatic reactions most amenable to therapeutic intervention.

Methods: In order to investigate the relationship between energy metabolism and cell division, we used a functional MRI approach to trace hyperpolarized [^{13}C]-pyruvate *in vivo* in a mouse model of liver regeneration at different time points after PHx (2 h, 24 h, 36 h, 48 h, and 96 h) selected in accordance to the transition of hepatocytes through the cell cycle. Gene expression was assessed using qPCR and WB.

Results: Cdk1$^{Liv-/-}$ mice displayed increased levels of alanine at 36 h and 48 h after PHx compared to their control littermates. This was consistent with an increased mRNA expression of *Gpt2* and *Got2*, two central enzymes in amino acid metabolism, gluconeogenesis and tricarboxylic acid (TCA) cycle. We also found a significant down regulation of *Idh1*, *Idh2* and *Idh3* from the TCA cycle, that regulates the generation of 2-oxoglutarate, a cofactor required for *Gpt2* to transform pyruvate to alanine, as well as the generation of the redox potential in the mitochondria. In order to understand the relationship between the increased alanine metabolism, the downregulation of genes related to the mitochondrial redox potential and cell division, we selected a set of genes involved in oxidation-reduction based on RNAseq data previously obtained at 96 h after PHx in Cdk1$^{Liv-/-}$ mice. Gene expression analysis confirmed that many genes from energy metabolism (i.e. *G6pdx*), carbon metabolism (i.e. *Mthfd1* and *Mthfd2)* fatty acid (*i.e. Abcd2*) and xenobiotic metabolism (i.e. *Ptgs*), displayed an opposite expression levels when compared cells that divide uncontrollably (samples from primary liver tumors) to cells that do not divide (samples from Cdk1$^{Liv-/-}$) suggesting that during cell division there are profound changes in the gene expression of metabolism.

Conclusions: The results from this preliminary study suggest that the block of cell division in the Cdk1$^{Liv-/-}$liver can induce a profound reprogramming in energy metabolism.

P0407
HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED EXTRACELLULAR VESICLES REVERSE HEPATIC STELLATE CELL ACTIVATION

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Background and Aims: Stem cells, and in particular induced pluripotent stem cells (iPSCs) represent a promising therapeutic approach for fibrotic diseases. Their use, however, is currently limited by issues such as scaling up production and eliminating cells with tumor-forming potential. Extracellular vesicles (EVs) are membrane surrounded structures released by cells, including iPSCs, and contain a footprint of the cell of origin. Here we tested the hypothesis that iPSC-derived EVs (iPSC-EVs) have an anti-fibrogenic effect on hepatic stellate cells (HSC), the key cell involved in liver fibrosis.

Methods: Human iPSC were generated as previously described (Israel MA et al., Nature, 2012). IPSC-derived EVs were isolated by differential centrifugation, quantified by FACS and characterized by dynamic light scattering and electron microscopy. Human HSC (LX2) were maintained in a quiescent-like phenotype by low-serum media and then treated with TGF-β (10 ng/mL) in presence or absence of iPSC-EVs or EV-free media for up to 16 h. Analysis of HSC phenotype, proliferation and chemotaxis were evaluated. Internalization of iPSC-EVs into HSC was assessed by fluorescent tracing techniques.

Results: Characterization of EVs identified microparticles (MP) as the main extracellular vesicle population released by iPSC. The fluorescent tracing assay detected iPSC-EVs internalized in HSC particularly after 6 h of incubation with EVs. The exposure of HSC to iPSC-derived EVs induced a 2-fold down-regulation of the pro-fibrogenic genes α-SMA, Collagen1α1 and TIMP-2 compared to cells treated with TGF-β and EV-free media ($p < 0.005$). A further analysis of the HSC responses occurring during fibrogenesis showed that iPSC-derived EVs reduced HSC proliferation and chemotaxis by 1.5-fold compared to TGF-β and MP-free media+TGF-β-treated HSC ($p < 0.05$). Previous studies have demonstrated that activated HSC may revert to an inactive phenotype (iHSC), which is similar but distinct from quiescence (qHSC). We observed that HSC exposed to iPSC-derived EVs share some quiescence-associated genes such as PPAR-γ, Nr1d2 and Foxj1 but showed a greater up-regulation of inactivation-associated genes, such as IL7R, Csf2rb and Ly86 compared to TGF-β-activated HSCs.

Conclusions: Our study uncovers iPSC-derived EVs as a potential anti-fibrotic therapeutic approach and demonstrates that they activate regenerative programs in HSCs by inducing a phenotypical switch from activated to their inactivated state.

P0408
THE REGULATION OF LIVER VOLUME GAIN AND REGENERATION-ASSOCIATED STEATOSIS THROUGH Pten

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Background and Aims: Successful liver regeneration is associated with the transient accumulation of lipids in hepatocytes, likely for the provision of energy to fuel the regenerative process. How this transient steatosis is regulated is ill-understood. The loss of Pten from liver induces rapid redistribution of peripheral fats into liver. Given that Pten also controls the growth-promoting Akt-mTORC1 pathway, Pten may act after resection to facilitate lipid import and to drive liver volume gain. To explore this possibility, we are analysing inducible, hepatocyte-specific *Pten* knockout mice following hepatectomy.

Methods: Pten levels were assessed in hepatectomized wild-type mice. *AlbCre$^{tg/+}$PTEN$^{fl/fl}$* and *AlbCre$^{+/+}$PTEN$^{fl/fl}$* (control) mice were studied shortly after tamoxifen induction. Regeneration was monitored via weight gain, proliferative activity and cyclin levels. Lipid content was quantified through a sulpho-phospho-*vanillin* protocol.

Results: In wild type mice, Pten levels dropped along with pronounced T389-Akt phosphorylation at 16 h post resection, coinciding with the peak of transient steatosis. In parallel, fatty acid import (Cd36) and β-oxidation (Cpt1a, Hadha/b) increased, whilst lipogenesis (Acc, Scd, Fasn) decreased. Inducing Pten loss In *AlbCre*tg/+PTENfl/fl mice resulted in spontaneous lipid accumulation. Two and three days after resection however, lipid content was similar to controls, yet liver weight gain was accelerated. Notably, the increased liver weight gain was not accompanied by elevations in proliferative markers, suggesting an involvement of hypertrophy.

Conclusions: Our results demonstrate a growth-promoting function for Pten downregulation during liver regeneration. The reduction in Pten may aid liver growth through (i) the release of inhibition of the hypertrophic Akt-mTORC1 pathway, and through (ii) the provision of energy via facilitated lipid import.

P0409

LKB1: A KEY REGULATOR OF HEPATOCYTES PROLIFERATION AND GENOME INTEGRITY

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Background and Aims: Liver Kinase B1 or LKB1 (also called Serine Threonine Kinase 11) is an evolutionarily conserved protein kinase which is involved in a diverse array of cellular processes, including energy homeostasis, cell polarity, apoptosis and cell growth. All these processes play a role in cancer initiation and progression. Their relative contribution to LKB1-mediated tumor suppression remains unclear. Interestingly, previous results have demonstrated that haploinsufficiency of LKB1 in the liver leads to the development of Hepatocellular Carcinomas. In the current study, we evaluate the potential effect of LKB1 deletion on hepatocyte proliferation.
Methods: We use hepatocyte specific LKB1 knockout mice to dissect the role of LKB1 in hepatocyte proliferation. We study the kinetics of liver regeneration after partial hepatectomy (PH). Livers (Control and KO) are dissected at different time points after PH in order to analyze DNA replication, division completion and various signaling pathways involved in hepatocyte proliferation.
Results: We first observe that following partial hepatectomy, LKB1 (−/−) hepatocytes exhibit early entry into S phase and prolonged proliferation leading to hepatomegaly. The priming events, lipid droplets accumulation and protein anabolic responses appear to be intact in LKB1 KO livers. Interestingly in KO hepatocytes, AMPK (AMP-activated protein kinase) is phosphorylated as cells proceed through S phase. Deletion of LKB1 impacts on G1/S transition by regulating the expression of key players involved in the initiation of S phase such as Cyclin A2, Cdc6 and E2F1. On the other hand, loss of LKB1 in hepatocytes also alters genomic integrity. LKB1 (−/−) hepatocytes present a block in metaphase/anaphase transition, associated with the increase of mitotic errors. A recent transcriptomic analysis highlighted the activation of different markers of the Spindle Assembly Checkpoint, such as Mad2 and BubR1. In line with these results, we observe that LKB1 deficient hepatocytes present an alteration of ploidy profile at the end of the regenerative process.
Conclusions: Collectively, these findings emphasize the key role of LKB1 in the regulation of hepatocyte proliferation and particularly on the maintenance of DNA and ploidy integrity.

P0410

CHARACTERIZATION OF THE SLOWLY CYCLING CELL POPULATION IN MOUSE LIVER

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Background and Aims: In many tissues, undifferentiated adult stem/progenitor cells are crucial for the tissue maintenance in normal conditions and for the recovery after injury. In the liver, however, the normal tissue turnover is achieved by the replication of its fully differentiated parenchymal cells. The existence of liver stem cells and their contribution in liver maintenance and regeneration is still under debate. Since tissue specific adult stem cells are considered to be slowly cycling or quiescent we used label retaining cell (LRC) assay to determine the localization and properties of potential liver stem cells.
Methods: Rosa26-rtTa mice that express ubiquitously expressed reverse tetracycline-dependent transactivator were crossed with H2B-GFP mice that express Histone 2B-enhanced green fluorescent protein (EGFP) fusion protein under the control of tetracycline response element to create double transgenic rtTa-GFP mice. For the induction of H2B-GFP expression (cell labeling) mice receive doxycycline (tetracycline analogue) in drinking water and after removing doxycycline from drinking water the dividing cells gradually lose their label whereas quiescent cells retain their GFP label (LRCs).
Results: We determined the optimal time for the admission of doxycycline, traced the label up to 20 weeks and identified the localization and the repertoire of markers of label-retaining GFP-positive cells under normal conditions. We discovered that slowly cycling liver cells concentrated mainly in the portal areas, particularly in bile ducts, and expressed biliary epithelial cell markers. We also found few LRCs among hepatocytes. These parenchymal LRCs, however, were individually scattered throughout the liver and had lower GFP expression. In addition, we studied the behaviour of LRCs in chronic liver injury and looked at the changes in LRC compartment after partial hepatectomy (PH) and CCl_4 intoxication. We found that proliferation of liver LRCs, indicated by the loss of GFP signal in biliary ducts, occurred after induced chronic injury (DDC diet and bile duct ligation). PH and CCl_4, on the other hand, did not affect the quiescent state of biliary LRCs, although we could detect less parenchymal LRCs after liver regeneration.
Conclusions: These results suggest that LRCs in bile ducts enter the cell cycle and take part in liver regeneration in response to chronic liver injury and only part of parenchymal LRCs contribute to the recovery from liver resection or CCl_4 intoxication.

P0411

PREGNANCY INDUCES SELECTIVE CHANGES IN HEPATIC GENES INVOLVED IN CELL PROLIFERATION AND APOPTOSIS IN MICE

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Background and Aims: During pregnancy the liver undergoes substantial structural and metabolic adaptations to meet the nutritional demands of the mother and fetus; however, the underlying mechanism is poorly understood. To address this, we analysed the expression of the liver transcriptome in non-pregnant and early, mid and late pregnancy mice.
Methods: C57/Blk6 Virgin mice (90 day old, $n \geq 6$) were mated and culled on d7, d14 or d18 gestation, or culled without mating. Livers were collected, weighed and snap frozen. Cell diameter was measured using haematoxylin and eosin (H&E). Proliferating cells were identified by immunohistochemistry using an anti-Ki67 antibody. Statistics were performed using either a one-way ANOVA with a Bonferroni *post hoc* test or Kruskal–Wallis with a Dunns *post hoc* test (threshold for significance $P < 0.05$). RNA was extracted and expression of the transcriptome was analysed using the Illumina MouseRef-8 v2.0 Expression BeadChip and analysed using IPA software (>2-fold changes in average signal were considered significant).
Results: There was no significant change in liver weight at d7, while liver weight at d14 was 20% ($P = 0.004$) and at d18, 60% ($P \leq 0.001$) greater compared to non-pregnant mice. This was accompanied by a 20% increase in cell diameter at d14 and 17% at d18 (both $P = 0.025$). The proportion of proliferating cells was unchanged at d7, but was increased at d14 and at d18 compared to non-pregnant liver. The expression of 115 genes was significantly altered (41 down and 74 up) at d14 and 123 (46 down and 77 up) at d18. Of these, 79 genes were changed at both time points. The top changed pathways at both d14 and d18 included small molecule biochemistry, molecular transport, organismal and tissue development. This reflected changes in genes consistent with increased cell proliferation at d14 and d18, and inhibition of apoptosis at d18 gestation.
Conclusions: These findings suggest that pregnancy-associated liver growth in mice is initiated between d7 and d14 of gestation.

This involves both hypertrophy and hyperplasia, accompanied by inhibition of apoptosis at d18. One possible interpretation is that the nutritional demands of the rapidly growing fetus in late gestation exceeds that which can be met by maternal liver growth mediated by cell proliferation alone and so may require decreased cell turnover in order to facilitate a sufficient increase in functional capacity.

P0412

NETRIN-1 PROTECTS AGAINST HEPATOCYTIC CELL DEATH UPON UPR THROUGH SUSTAINED TRANSLATION IN AN UNC5A/C-DEPENDENT FASHION

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Background and Aims: The unfolded protein response (UPR) is a hallmark of viral and non-viral hepatic diseases and is involved in hepatic oncogenesis. Netrin-1 is an anti-apoptotic secreted factor up-regulated in many cancer types in which chronic Endoplasmic Reticulum (ER) stress is observed. To restore ER homeostasis, cells activate UPR, which may also trigger or impede apoptosis through different processes including DAPK activation. In hepatocellular carcinoma, cells are resistant to UPR-induced apoptosis, prompting us to study the anti-apoptotic role of Netrin-1 upon UPR in the hepatic context.
Methods: UPR was experimentally induced by DTT and tunicamycin in vitro (Huh7.5 cells, HepaRG cells, primary human hepatocytes) and in WT or Netrin-1-overexpressing mice, respectively. Netrin-1 translation was studied through polysome isolation in vitro, in mice, and in liver resections. Netrin-1 5′UTR was studied by SHAPE and DMS and its ability to recruit the ribosomal 40S subunit. Apoptosis induction was monitored after RNAi depletion or forced expression of Netrin-1 and various effectors of the Netrin-1/UNC5 pathway through DNA fragmentation, caspase-3 and PP2A activities in vitro and in mice.
Results: Functional UPR activation, as determined by GRP94, CHOP, and XBP1s mRNA expression, was verified in all systems. SHAPE and DMS experiments showed that Netrin-1 5′UTR bears highly structured motifs and autonomously recruits ribosomal 40S subunits. Netrin-1 mRNA remained strongly associated with translational units upon global shut-down of protein translation caused by UPR compared to the cap-dependent translated Gus and β-actin mRNAs in HepaRG hepatocyte-like cells, Huh7.5 hepatoma cells, mice livers, and in UPR(+) patient resections. In the absence of Netrin-1, Netrin-1 receptors UNC5A and C trigger apoptosis through activation of the DAPK1/PP2A/PR65b complex. Netrin-1 overexpression in mice protected against UPR-induced cell death as evaluated by caspase-3 and TUNEL assays.
Conclusions: These results indicate that Netrin-1 (i) is resistant to global translational repression upon UPR in hepatocytes, probably through IRES-dependent mechanisms, (ii) could protect cells against UPR-related cell death after binding to UNC5A and C, leading to the inhibition of DAPK1-mediated apoptosis and (iii) may condition the overall survival rate of hepatocytic cells upon UPR, a frequently activated cellular function in the context of hepatocarcinoma-associated chronic liver diseases.

P0413

D-DOPACHROME TAUTOMERASE: NOVEL REGULATOR OF HEPATIC AUTOPHAGY

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Background and Aims: Characterized as an adipokine, d-dopachrome tautomerase (DDT) also functions as a ligand for CD74 signaling, and is expressed in various other tissues including liver. Examination of array/qPCR data showed DDT to be significantly increased in livers of high-fat diet fed mice compared to controls. The Comparative Proteomic Analysis Software Suite database indicated DDT interacts with several proteins involved in cell metabolismand autophagy, both of which are known to be disturbed in high-fat diet-induced NAFLD. The aim of this study was to explore a possible link between dysregulated DDT expression and liver cell function.
Methods: Mouse hepatocytes, AML12 and Hepa1–6 cells were used in siRNA-mediated knockdown experiments. Vesicle formation was monitored via bright-field and fluorescent microscopy. Gene expression was analyzed by qPCR and/or immunoblot. For analysis of autophagosomes, cells were transduced with a GFP-LC3B tandem autophagy sensor via baculovirus.
Results: qPCR revealed higher expression of DDT in AML12 cells and primary hepatocytes compared to Hepa1–6 cells. Various assays were performed to assess cellular function following DDT knockdown. While slight impairment of proliferation was observed, the most striking effect was formation/accumulation of cytoplasmic vesicles. Vesicle accumulation was consistent between primary hepatocytes and AML12 cells, but absent in Hepa1–6 cells. Interestingly, treatment with forskolin led to a decrease in the number and size of vesicles, which was absent in controls. As the structures induced by DDT knockdown had lipid droplet-like morphology, staining for neutral lipids was performed. Two independent staining approaches did not stain these structures, indicating the vesicles do not contain neutral lipids. However, treatment of AML12 cells with an autophagy inhibitor, chloroquine phosphate, led to an induction of vesicles in the cytoplasm resembling those in DDT knockdown cells. Evaluation of various autophagy markers showed dysregulated p62, ATG5 and ATG7 in DDT knockdown cells. Finally, with use of the LC3B tandem sensor, vesicles were marked as inhibited autophagosomes.
Conclusions: Overall, knockdown of DDT appears to inhibit autophagy in vitro, with inhibition characterized by accumulation of ATG5 and p62 leading to the formation of autophagosomes, which do not fuse with lysosomes. These data are the first to indicate DDT may have a functional role in hepatic autophagy, a novel link that warrants further investigation.

P0414

CONSTITUTIVE ANDROSTANE RECEPTOR-MEDIATED DOWN-REGULATION OF MIR-122 IN LIVER

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Background and Aims: MiR122 is a major hepatic miRNA, accounting for more than 70% of total liver miRNA population. MiR122 is involved in hepatocyte proliferation, differentiation, apoptosis and associated with liver diseases, including HCC. Mir122 is an intergenic miRNA with its own promoter. Pri-miR122 expression is regulated by liver enriched transcription factors, mainly by HNF-4a, which mediates expression via interaction with

DR1 site. Phenobarbital-mediated constitutive androstane receptor (CAR) activation is associated with the decrease of miR-122 level in liver. CAR can modulate pathways involved in liver regeneration after PH, induce direct hyperplasia and has profound effects on hepatic carcinogenesis. CAR can inhibit HNF4a transcriptional activity by competing with HNF4a for binding to the DR1 motif in gene promoters. In present study we investigated HNF4a-CAR crosstalk in the regulation of the hepatic miR122 level.
Methods: Mice were treated ip with TCPOBOP and/or 5α-androstan-3α-ol (Andr). Gene expression levels were measured by real-time PCR. Western blot analysis was performed to determine any changes in proteins. ChIP assay was performed on the chromatin extracted from livers to demonstrate the recruitment of transcription factors to their respective binding sites on the gene promoters.
Results: The level of miR122 was significantly repressed by treatment with TCPOBOP, which is an agonist of mouse CAR. In order to assess the involvement of the CAR on the down-regulation of miR122, the TCPOBOP treatment was repeated in the presence of CAR inverse agonist Andr. When Andr was given prior to TCPOBOP, it caused an increase of the hepatic levels of miR122 compare to TCPOBOP treated only. ChIP assays demonstrated that TCPOBOP-activated CAR inhibited HNF4a transactivation by competing with HNF4a for binding to DR1 site in pri-miR122 promoter. Transcription factors replacement was strongly correlated with miR122 down-regulation. CAR interaction with DR1 site was abolished after Andr co-treatment. CAR-mediated down-regulation of miR122 was correlated with up-regulation of its targets, including Cyclind1, E2f1 and E2f1-target gene, cMyc, which promote G1/S cell cycle progression. Long-term TCPOBOP treatment caused a significant increase in both the liver mass and liver-to-body weight ratio.
Conclusions: Thus, our results provide evidence to support the conclusion that CAR activation decreases miR122 level through suppression of HNF4a transcriptional activity.

P0415
CELL CYCLE REGULATION IS DELAYED IN A MODEL OF LIVER REGENERATION AFTER SERIAL PARTIAL HEPATECTOMY

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Background and Aims: Hepatocytes enter the cell cycle to recover the original liver mass, when confronted with liver damage. Since little is known about the condition of the liver after it has regenerated, here we describe a new model to study the regulation of the cell cycle in a mouse model of serial partial hepatectomy (PHx).
Methods: Serial PHx consists of the subsequent removal of different liver lobes: first the median and left lobes (PH1), 2 weeks later the superior right lobe (PH2) and 2 more weeks later, the inferior right lobe (PH3). Livers were collected at 24, 48, 72, and 96 h after each PHx. These time points correspond to the transition from G1 to S phase, from G2 to mitosis, and the transition through mitosis, respectively. Analysis was done by IHC, WB, and RT-PCR.
Results: BrdU incorporation, a marker of DNA replication in S phase, was decreased from 25% $BrdU^+$ cells at 48 h after PH1 to 20% after PH2 and to 15% after PH3. While the peak of BrdU incorporation was observed at 48 h for PH1 and PH2, it was delayed to 72 h after PH3. Interestingly, a peak of 60% of $Ki67^+$ hepatocytes was observed at 72 h after PH1 and a peak of 35% after PH2, with a gradually decrease over time. However, 65% of $Ki67^+$ hepatocytes was already observed at 48 h after PH3 and remained at that level until 96 h. These results suggest that the progression through the cell cycle could be affected by serial PHx, with a decrease in the number of cells transiting through the cell cycle after PH2 and a delay in the timing of the transition through the cell cycle after PH3. Our study of gene expression revealed an important decrease after PH2 and PH3 in the level of cyclin A1 and A2 as well as cyclin B1, B2, B3 and Cdk1, controlling the transition through G2 and cell division, respectively. A significant downregulation was detected in the levels of cyclin D1 after PH2 and PH3, suggesting that there may be a defect in the regulation of signalling pathways in early phases of liver regeneration. Finally, while Cdk4 and Cdk6 did not display significant changes, the expression of Cdk2 was slightly delayed after PH3 only, indicating that other earlier phases of cell cycle progression may not be affected.
Conclusions: Our study indicates that up to 90% of the liver can be resected using the serial PHx protocol with a high viability of the mice. Nevertheless, cell cycle regualtion in the different hepatectomies seem to differ in their molecular mechanism, which will need to be studied in more details in the future.

P0416
RELEVANCE OF BMP9-MEDIATED SIGNALING IN OVAL CELL FUNCTION DURING LIVER INJURY. CROSSTALK WITH THE HGF/Met PATHWAY

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Background and Aims: Oval cells constitute a bi-potential progenitor cell population from adult liver. Under chronic liver disease, they become activated and start to proliferate and differentiate into cholangiocytes and/or hepatocytes to compensate for the cellular loss and contribute to maintain liver homeostasis. However, some evidences support a pro-fibrogenic and pro-tumorigenic role for these cells. BMP9, a member of the BMP family, has recently emerged as a critical regulator of liver pathology. Specifically, results from our group and others have evidenced a pro-tumorigenic action of BMP9 in HCC cells. This prompted us to explore the potential role for BMP9 in regulation of liver progenitor cells function.
Methods: For that, we have used oval cell lines harboring a genetically inactivated met tyrosine kinase ($Met^{-/-}$ cells) and their control (Metflx/flx cells) that allow us to explore a potential signaling interaction between HGF and BMP9. We show that BMP9 induces Smad1/5/8 signaling in both Metflx/flx and $Met^{-/-}$ cells, but interestingly, Met significantly potentiates BMP9-induced signaling. To understand how this affects cell response to BMP9, we run functional assays.
Results: Our results indicate that BMP9 elicits a decrease in cell number, which is associated with induction of a moderate but significant apoptotic response. This effect is consistently more pronounced in $Met^{-/-}$ cells. Importantly, HGF is able to prevent BMP9 effects. In addition to acute BMP9 treatment, we have submitted oval cells to chronic treatment with BMP9. Phenotypic and functional characterization of these cells (named as B9T-OC) has evidenced an up-regulation of epithelial and hepatic lineage markers (E-cadherin, HNF4a and albumin) and concomitant loss of mesenchymal and hematopoietic stem cell markers.
Conclusions: Altogether, our findings reveal a novel role for BMP9 as an important regulator of oval cell phenotype and survival. While acute BMP9 treatment elicits a suppressor activity, chronic treatment promotes acquisition of a more epithelial and differentiated phenotype. Additionally, we evidence a novel

functional crosstalk between HGF/Met and BMP9 pathways in regulation of oval cell survival, whose biological significance has not yet been clarified. In vivo studies, using a model of chronic liver damage associated with expansion of oval cells, are being conducted in wild type and BMP9 knockout mice. They will help us to further clarify the role played by BMP9 in liver injury and how it may determine oval cell fate.

Molecular and cellular biology: b. HSCs and Fibrosis

P0417
Nlrp3 INFLAMMASOME INCREASES HEPATIC FIBROSIS BY INDUCING INFLAMMATORY SIGNALS IN HEPATIC STELLATE CELLS

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Background and Aims: Hepatic fibrosis represents the wound-healing response process to chronic liver injury, independently from aetiology. Hepatic stellate cells (HSCs) are the main liver extracellular matrix producing cells and also exert proinflammatory activity. The NLRP3 inflammasome mediates the release of proinflammatory cytokines in response to cellular danger signals in various organs, but its role in the progression of hepatic injury has to be determined. Thus, aim of the study was to evaluate the role of NLRP3 inflammasome in fibrosis development and in HSCs behaviour.
Methods: Wild-type (WT) C57BL/6 and *Nlrp3*^{A350VneoR} (*Nlrp3*$^{-/-}$) mice underwent bile duct ligation (BDL) and were sacrificed at 3 weeks. Quiescent HSCs (quHSCs) from WT mice were obtained after 12 hours of culture, while *in vitro* activated HSCs (AcHSCs) were obtained after 6 days of culture.
Results: Compared to WT, *Nlrp3*$^{-/-}$ BDL mice showed a significant reduction in inflammasome activation in the liver (Caspase-1, IL1β and IL18 gene expression) that was associated to significantly decreased hepatic TLR4, TLR9 and IL6 mRNA levels. Type I collagen, TGFβ, CTGF and TIMP1 gene expression were significantly reduced in *Nlrp3*$^{-/-}$ BDL mice. This led to reduced collagen deposition measured by Sirius Red staining in *Nlrp3*$^{-/-}$ BDL mice. *In vitro* HSCs activation was associated to significantly decreased gene expression of inflammasome components (NLRP3/ASC/Caspase-1/IL1β/IL18) compared to (qu)HSCs. However, (Ac)HSCs stimulation with the pathogen-associated molecular pattern LPS induced expression of the NLRP3/Caspase-1/IL1β/IL18 pathway, without affecting neither type I collagen mRNA nor cell proliferation. After priming with LPS, incubation with the damage-associated molecular pattern ATP was needed for the release of IL1β. (Ac)HSCs incubation with recombinant IL1β further stimulated the NLRP3/Caspase-1/IL1β pathway and lead to increased TGFβ and CTGF gene expression and HSCs migration.
Conclusions: NLRP3 inflammasome contributes to hepatic fibrosis by increasing the inflammatory response (increased IL1β and IL18 gene expression) and inducing a paracrine loop that maintains TLRs activation and IL6 production. IL1β released by (Ac)HSCs stimulates fibrogenesis and cell migration in an autocrine manner. Several new therapeutic targets can be identified in the NLRP3 inflammasome pathway to treat hepatic fibrosis.

P0418
GENERATION AND CHARACTERIZATION OF A MYOFIBROBLASTS SPECIFIC CONDITIONAL Col3a1a-CreERT2 MOUSE MODEL TO STUDY LIVER FIBROSIS

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Background and Aims: Activated myofibroblasts play a central role in liver fibrosis via expression of most of the scar tissue that characterizes fibrosis and cirrhosis. While collagen type I is mainly expressed in bone, collagen type III is largely restricted to soft tissue (myo-) fibroblasts Moreover, for studying gene functions in *vivo*, models for specific target inducible gene disruptions using cell type-specific gene promoters are needed. Therefore, we aimed to generate a mouse line with an inducible (myo-) fibroblast specific Col3a1 promoter controlling Cre recombinase expression.
Methods: A 204 kb BAC from a genomic library (Children's Hospital Oakland Research Institute) that contains the full-length mouse Col3a1 gene (RP23–324D9) was selected for recombineering in a EL250 bacteria targeting cassette encoding CreERT2 and a kanamycin resistance gene flanked by two FRT sites. This construct was integrated into the ATG-start codon of the Col3a1 gene by homologous recombination. The FRT-flanked kanamycin resistance cassette was subsequently deleted by arabinose-induced expression of Flp recombinase. The purified, linearized Col3a1-CreERT2 BAC DNA fragment was microinjected into the pronucleus of C57BL/6N oozytes. Transgenic founder mice were identified by PCR genotyping of tail tips.
Results: To test the CreERT2 mouse line, a double-fluorescent Cre reporter mouse that expresses membrane-targeted Tomato prior to Cre-mediated excision and membrane-targeted green fluorescent protein after excision was used. Bi-transgenic Col3a1-CreERT2/Tomato mice were treated with CCl_4 for three weeks, to induce liver fibrosis and with tamoxifen for the last five days, to induce Cre recombination. Thereafter, mice were analyzed *in vivo* using confocal laser scanning microscopy showing a massive GFP signal compared to control animals without tamoxifen treatment. Dual-label fluorescent immunohistochemistry using liver sections demonstrated perfect co-localization of GFP positive cells with procollagen a1(III) stained areas and of GFP with a-SMA, indicating that all GFP positive cells were activated myofibroblasts.
Conclusions: We generated a conditional Col3a1a-CreERT2 mouse that promises to be highly useful for the study of myofibroblast activation, gene expression and antifibrotic target discovery in liver fibrosis.

P0419
IN VIVO CELL SPECIFIC GENE SILENCING IN THE LIVER USING NOVEL SIRNA-LOADED NANOHYDROGEL PARTICLES

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Background and Aims: Efficient *in vivo* transport of active siRNA to specific liver cells remains challenging. Compared to lipoplex or polyplex formulations, cationic nanohydrogel particles (NHP)

can serve as superior oligonucleotide carriers [Siegwart DJ et al, PNAS 2011]. We have designed NHP [Nuhn L et al, ACS Nano 2012] that serve as stable carriers for siRNA, avoid carrier aggregation under physiological conditions and promote size-dependent gene silencing *in vitro* [Nuhn L et al, Biomacromolecules 2014].

Results: NHP composed of block copolymers [pentafluorophenyl methacrylate and tri(ethylene glycol) methyl ether methacrylate] loaded with negative control siRNA up to 600 nM siRNA concentrations which are far above concentrations for *in vivo* knockdowns and of a size of 30 to 40 nm did not show cytotoxicity for murine 3T3 fibroblasts, Raw or MHS macrophages, or for HepG2 human hepatoma cells and AML-12 murine benign hepatocytes. In media with 10% fetal calf serum FITC-labelled NHP were taken up efficiently and dose-dependently by all cells tested, reaching up to 90% after 1 h incubation as determined by FACS analysis. NHP loaded with procollagen a1(I) or CD68 siRNA yielded a robust knockdown in 3T3 and Raw cells, respectively, after 48 h of incubation as determined by qPCR.

In vivo studies using near infrared labelled NHP loaded with Cy5 labelled procollagen a1(I) siRNA were performed in mice with CCl_4-induced liver fibrosis [Popov Y et al., Hepatology 2009]. After intravenous injection NHP distributed preferentially to the liver (80%), as determined by *in vivo* and *ex vivo* near infrared imaging. Double labelling immunohistochemistry and *ex vivo* FACS analysis revealed a preferential colocalization with myofibroblasts (α-SMA positive) and macrophages (CD45+, F4/80+, CD11b+). NHP loaded with procollagen a1(I) siRNA generate an up to 80% *in vivo* knockdown in CCl_4-induced liver fibrosis. Efficient knockdown was further confirmed by decreased liver collagen (Sirius red staining and hydroxyproline content).

Conclusions: siRNA loaded NHP accumulate in liver and especially in (myo-) fibroblasts and macrophages/Kupffer cells. Due to their intrinsic specificity for nonparenchymal cells, an apparent lack of toxicity and a high loading capacity, they are attractive carriers for siRNA-based antifibrotic or immune modulatory therapies.

P0420
SELECTIVE INHIBITOR OF WNT/β-CATENIN/CBP SIGNALING AMELIORATES HEPATITIS C VIRUS-INDUCED LIVER FIBROSIS IN MICE

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Background and Aims: Chronic hepatitis C virus (HCV) infection is one of the major causes of developing serious liver diseases, including liver cirrhosis and hepatocellular carcinoma (HCC). An estimated with one million people die from cirrhosis worldwide each year. Unfortunately, at present there are not-sufficient effective anti-fibrotic drugs for liver cirrhosis. In this study, we investigated the anti-fibrotic activities of PRI-724, a selective small molecule inhibitor of β-catenin/CBP (cyclic AMP response element binding protein) interaction, using hepatitis C transgenic mouse model mimicking chronic HCV infection, which induced fibrosis.

Methods: The anti-fibrotic activity of PRI-724 was assessed by using HCV GT1b transgenic mice at 18 months after HCV genome expressing, which mimic chronic HCV infection and develop liver fibrosis and HCC. PRI-724 was continuously injected with mice for 6 weeks using osmotic pump. We observed histological features, serum ALT activity, HCV core protein level, the number and characteristics of the intrahepatic leukocytes. Liver fibrosis was monitored by histology with Silver stain, Masson trichrome, Sirius red staining and quantification of hepatic hydroxyproline amounts. Furthermore, mRNA expression levels in the liver following PRI-724 treatment were analyzed by quantitative real-time PCR.

Results: Continuous subcutaneous infusion of PRI-724 (1 mg/kg/day) for 6 weeks reduced collagen fibrils and collagen levels in the liver as indicated by Silver stain, Masson trichrome, Sirius red staining and quantification of hydroxyproline levels, respectively. There was no change in ALT levels after treatment, suggesting that anti-fibrotic effect of PRI-724 was not caused by cytotoxic or cytolytic activities. We found that although the MMP-8 (collagenase-2) and MMP-9 mRNA expression in the liver of HCV Tg mice was suppressed, PRI-724 administration induced both MMPs mRNA expression in the liver. Consistent with this result, we showed that intrahepatic neutrophils and macrophages increased in the liver after PRI-724 injection.

Conclusions: PRI-724 ameliorated HCV-induced liver fibrosis in mice model. MMP-8 and MMP-9 induction might contribute to the resolution of fibrosis by PRI-724. Thus, we suggested that PRI-724 would be one of the candidates of anti-fibrotic drugs for HCV-induced liver fibrosis.

P0422
REINTRODUCTION OF miR19b INHIBITS HEPATIC STELLATE CELL-MEDIATED FIBROGENESIS

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Background and Aims: Hepatic stellate cell (HSC) activation mediated by transforming growth factor beta (TGFb) is a key event in hepatic fibrosis. MicroRNA 17–92 (miR17–92) cluster consisting of miR17a-18a-19a-20a-19b and 92 modulate components of the TGFβ pathway. MiRs in this cluster are differentially expressed during liver injury, with miR19b significantly downregulated. Further, miR19b is a regulator of TGFb signaling in HSCs. Several mRNAs critical to HSC activation and TGFb signaling, such as TGFb receptor II (TGFbRII), are miR19b targets. We *hypothesize* that HSC-targeted reintroduction of miR19b by adeno-associated virus, serotype 2 (AAV2), will inhibit fibrosis during hepatic injury.

Methods: Culture activated (day 10) primary Sprague-Dawley rat HSCs, isolated by standard pronase/collagenase perfusion, were used. HSCs were treated with tyrphostin-1 (500μM, 4 hours), media changed, and cells transduced with AAV2 (5000 MOI) containing GFP with mature-miR19b (AAVmiR) or truncated miR19b control (AAVcon) under transcriptional regulation of the collagen α1(I) promoter for 24 or 48 hours. Additionally, Sprague-Dawley rats underwent sham or bile duct-ligation (BDL). AAVmiR or AAVcon (5×10^{11}, 1×10^{12}, or 2×10^{12} VG/rat) was injected via portal vein at the time of BDL/sham surgery. Tissues were harvested at necropsy 1 or 2 weeks post BDL/AAV2 surgery. In both models, pro-fibrotic gene expression and markers of HSC activation were measured by qRT-PCR and Western blot. AST/ALT levels in rat plasma and collagen deposition in FFPE liver sections were also examined.

Results: *In vitro*, pro-fibrotic mRNA and protein expression [αSMA, colα2(I), and TGFbRII] were significantly downregulated in AAVmiR transduced HSCs compared to AAVcon. Transduction was confirmed by viewing GFP positive cells by fluorescent microscopy. *In vivo*, hepatic collagen deposition was decreased by 80% in BDL/AAVmiR *vs* BDL/AAVcon treated animals, despite the obvious injury induced by BDL. AST/ALT levels were also reduced. Further, pro-fibrotic mRNA and protein expressions were significantly decreased in AAVmiR animals. Expression of miR19b increased in a dose dependent manner in AAVmiR treated animals.

Conclusions: AAV2 reintroduction of miR19b ameliorated fibrosis by inhibiting fibrotic gene expression and HSC activation, which correlated with increased miR19b expression. These data support HSC-targeted reintroduction of miR19b as an effective anti-fibrotic therapy.

P0423

ROLE OF APOPTIC DNA AND EXTRACELLULAR CORE HISTONES IN INFLAMMASOME ACTIVATION IN PRIMARY HUMAN HEPATIC STELLATE CELLS

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Background and Aims: Hepatocyte cell death is accompanied by extensive release of cleaved genomic DNA (mono- and polynucleosomes) in the extracellular compartment. As sustained hepatocyte cell death is implicated in the development of hepatic fibrosis, portal hypertension and liver cancer, this study aimed to investigate the effect of nucleosomes (from apoptotic hepatocytes) and extracellular histones on human Hepatic Stellate Cells (hHSC) and their role in inflammasome activation.
Methods: Cleaved nucleosomes were obtained (a) from apoptotic hepatocytes and (b) by nuclease digestion of intact nuclei. Nucleosome-associated DNA was purified. Core histones (H3 and H4) were purchased. Primary hHSCs were cultured in serum-enriched growth medium followed by serum deprivation for 24-hours before treatments. Dose-dependent responses of hHSC cells to uncleaved, cleaved nucleosomes and purified DNA (0.5–2 μg/ml range) and to purified extracellular histones (5–50 μg/ml) were tested. Cell viability (MTS assay) and IL1β/IL18 secretion (hIL1β/18 reporter cells) were tested. TLR4 transactivation by histones was evaluated using hTLR4 reporter cells.
Results: Treatment with mono- and polynucleosomes derived from hepatocytes induced early and progressive dose-dependent cell injury and death of the primary hHSC. Neither the uncleaved nucleosomes nor the purified nucleosome-associated DNA had any effect. To test whether the core histones could be the mediators of the observed response to cleaved nucleosomes, HSCs were treated with both a mixture of histones and H3 and H4 isoforms; a dose-dependent change in cell morphology but not in viability was observed, mostly in response to H3. As HSCs express TLR4, we tested if histones induced transactivation of TLR4, leading to inflammasome activation and IL1β/IL18 secretion. Exposing hTLR4 reporter cells to histones led to a dose-dependent response (20-fold, with highest dose of H3). Treatment of hIL1β/18 reporter cells with supernatants from histone-treated hHSCs, resulted in a significantly higher transactivation.
Conclusions: These data show for the first time that the core histones (most potently H3), but not the DNA, in the cleaved nucleosomes from apoptotic hepatocytes affect primary hHSC cell biology in vitro and induce a proinflammatory response probably through TLR4-signaling. Therefore targeting extracellular histones and/or TLR4 signalling could result in attenuation of hHSC activation and inflammation associated with liver injury.

P0424

DELIVERY OF RELAXIN ATTENUATES ESTABLISHED LIVER FIBROSIS BY SUPPRESSING COLLAGEN CROSS-LINK AND ENHANCING COLLAGEN DEGRADATION

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Background and Aims: Liver fibrosis is characterized by excess accumulation and repressed degradation of extracellular matrix. While means of alleviating already established liver fibrosis has scarcely been reported, relaxin is showing some promising results. In this study, we investigated whether a single adenoviral delivery of relaxin gene would attenuate established liver fibrosis in rats.
Methods: Liver fibrosis induced by 8 weeks of thioacetamide (TAA) treatment is known to persist without recovery at least 2 months. Rats were infected once with either relaxin-expressing adenovirus (TAA+Relaxin) or control virus (TAA+Vector) via tail vein after 8 weeks of TAA treatment. They were sacrificed either 3 days or 3 weeks after the adenovirus infection.
Results: Morphometric analysis of sirus red stained area demonstrated that TAA+Relaxin group had significantly decreased fibrosis at 3 weeks after the virus delivery although this change was not prominent at 3 days after the treatment. The assessment of *collagen α1(I)* [*Col1α(I)*] expression agreed with the histological evaluation. Expression of relaxin receptor (*Rxfp1*, *Relaxin-like family peptide receptor-1*) increased in both TAA+Vector and TAA+Relaxin rats at 3 days after the delivery, although TAA+Relaxin group had more prominently increased *Rxfp1*. After 3 weeks, TAA+Vector rats had reversed *Rxfp1* expression when TAA+Relaxin rats still had increased *Rxfp1* level. Liver *α-smooth muscle actin* (*α-SMA*) mRNA in both TAA+Vector and TAA+Relaxin rats was increased after 3 days but reversed to the normal level after 3 weeks. Analysis of *TGFβ* and *MMP9* mRNA demonstrated the similar pattern. Expression of *lysyl oxidase homolog 2* (*LOXL2*), which is crucial in the formation collagen cross-links, was increased in TAA+Vector and TAA+Relaxin rats at 3 days after virus treatment. Although both groups had attenuated *LOXL2* expression after 3 weeks, TAA+Relaxin group had further decreased *LOXL2* expression compared to that of the normal liver. Expression of tissue inhibitor of metalloprotiase-2 (TIMP-2), that prevent collagen degradation, was elevated in both groups at 3 days after the virus treatment. While TAA+Vector group still had sustained elevation of TIMP-2 expression, TAA+Relaxin rats had suppressed TIMP-2 expression after 3 weeks.
Conclusions: A single delivery of relaxin gene in the liver attenuated established hepatic fibrosis by suppressing collagen cross-links and enhancing collagen degradation.

P0426

INHIBITION OF MONOACYLGLYCEROL LIPASE ACCELERATES LIVER FIBROSIS REGRESSION

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Background and Aims: Monoacylglycerol lipase (MAGL) is the rate-limiting enzyme in the degradation of monoacylglycerols, which are short-lived intermediates of lipid catabolism. MAGL is also a pivotal component of the endocannabinoid system, since this enzyme metabolizes 2-arachidonoyl-glycerol, an endocannabinoid receptor ligand, into arachidonic acid. Recent studies have identified inhibition of MAGL as an interesting anti-inflammatory strategy in several experimental models of chronic inflammatory diseases. In the present study, we investigated the impact of MAGL inhibition on liver inflammation and fibrosis regression.
Methods: Liver fibrosis regression was studied in C57BL/6 mice exposed to chronic administration of carbon tertrachloride (CCl_4) for 6 weeks. Injections were discontinued and mice were daily administered with the MAGL inhibitor JZL184 (15 mg/kg, i.p.) or vehicle for up to 4 days. In vitro studies were performed on isolated murine peritoneal macrophages and hepatic myofibroblasts, and on PBMC from alcoholic cirrhotic and healthy subjects. Sirius red staining was quantified by morphometry. Fibrogenic and inflammatory gene expression was analyzed by RT-PCR. The identity of intrahepatic leucocytes were analyzed by FACS.
Results: JZL184 accelerated fibrosis regression 4 days after cessation of CCl_4 administration, as shown by a reduction of sirius red

staining and smooth muscle alpha actin immunolabeling, and decreased expression of fibrogenic genes in treated vs untreated animals. Moreover, JZL184 significantly decreased the influx of Ly6C+ infiltrating monocytes into the liver of CCl_4-exposed mice. Accordingly, JZL 184 reduced the hepatic expression of Ly6C mRNA and decreased the hepatic expression of IL1-β, CCL2, CCL4 and CCL5 mRNAs. In vitro studies demonstrated that JZL184 (i) reduced hepatic myofibroblast proliferation; (ii) down-regulated LPS-stimulation of inflammatory mediator expression (IL1-β, CCL2, CCL4 and CCL5 mRNAs) from peritoneal macrophages. Finally, MAGL mRNA expression was increased in PBMC from patients with alcoholic cirrhosis as compared to healthy subjects and further enhanced in LPS-exposed PBMC.
Conclusions: Pharmacological inhibition of MAGL accelerates liver fibrosis regression in a model of toxin-induced liver injury, most probably by a mechanism involving reduction of infiltrating pro-inflammatory monocytes into the liver and a decrease in inflammatory mediators produced by macrophages. These results unravel MAGL inhibition as a novel promising antifibrogenic approach.

P0427
PLATELET-DERIVED GROWTH FACTOR-D INTENSIFIES FIBROGENESIS THROUGH UPREGULATION OF TIMP-1 AND SIGNALING VIA BOTH PLATELET-DERIVED GROWTH FACTOR RECEPTOR TYPE ALPHA AND BETA
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Background and Aims: Platelet-derived growth factor-D (PDGF-D) is a more recent recognized growth factor involved in the regulation of several cellular processes, including cell proliferation, transformation, invasion, and angiogenesis by binding to and activating its cognate receptor PDGFR-β. In the experimental liver fibrotic models BDL and CCl_4, PDGF-D showed upregulation comparable to PDGF-B. Moreover, adenovirus PDGF-D gene transfer induced hepatic stellate cell proliferation and liver fibrosis. We now seek to further uncover the molecular mechanism of PDGF-D involvement in liver fibrogenesis.
Methods: PDGF-D stimulation in profibrogenic primary cells of portal myofibroblasts (pMF), hepatic stellate cells (HSC) were evaluated for fibrogenic markers and PDGF-D signaling pathways compared to the other PDGF isoforms.
Results: We found PDGF-D not to enhance collagen type I and a-SMA production, but instead to significantly upregulate TIMP-1 expression in both mRNA and protein levels, resulting in attenuation of MMP2 and MMP9 gelatinase activity as indicated by gelatinase zymography. Unexpectedly, PDGF-D incubation did decrease both PDGFR-α and -β in mRNA and protein levels, and PDGF-D did phosphorylate Tyr 754 and 1018, the specific Tyr for PDGFR-α. Tyr phosphorylations from PDGF-D stimulation showed identical to that of PDGF-B. For the first time we could also demonstrate that PDGF-D binds to the recombinant PDGFR-α-FC homodimers.
Conclusions: PDGF-D intensifies fibrogenesis by interfering with the fibrolytic activity of the TIMP1/MMP system and PDGF-D signaling through both PDGF-α and -β receptors.

P0428
LPS-STIMULATED MOUSE HEPATIC STELLATE CELLS SECRETE SPECIFIC FACTORS THAT DIRECTLY CONTRIBUTE TO THE ACUTE PHASE RESPONSE OF HEPATOCYTES
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Background and Aims: The acute phase response (APR) is a systemic inflammatory condition initiated by activated phagocytic cells, fibroblasts and endothelial cells to prevent tissue damage and initiate repair processes.
The aim of our study was to determine the contribution of hepatic stellate cells (HSC) towards the APR induction. To this end, we quantified the secretory output of HSC with respect to the main acute phase cytokines. Hepatocytes' response to the secretory HSC output was analysed by Fluidigm real time PCR analysis.
Methods: Primary mouse HSC were cultivated for 48 hours before adding LPS to the medium. The release of 23 cytokines and chemokines was analysed by Luminex cytokine array technology after 24 hours of LPS stimulation (50 ng/ml). The main acute phase cytokines IL6, IL1-beta and TNF-alpha were further quantified after 2, 6 and 24 hours of LPS stimulation (0/10/50/100 ng/ml).
Tissue culture supernatant of HSC following 24 hours stimulation with 100 ng/ml LPS was used to stimulate primary mouse hepatocytes for 24 hours. Expression of 48 acute phase proteins (APP) in hepatocytes was analysed by Fluidigm real time PCR analysis and compared to hepatocytes treated directly with LPS or IL6.
Results: In HSC, G-CSF, KC (Cxcl1), IL-12 (p70), MCP-1a (Ccl2), TNFα and MIP1a (Ccl3) were the most prominently and significantly upregulated secreted cytokines after 24 hours and transcription of Ccls -2, -3, -4, -5 and -7, as well as Cxcls -1, -2, -3 and -5 were upregulated by LPS together with IL1b, IL6 and TNFa in HSC. Stimulation of hepatocytes with conditioned medium from LPS-treated HSC led among others to increased mRNA levels of Saa1, Saa2 and Orm1. Other targets like Hepcidin, Saa3 and IL-33 were already upregulated by direct treatment with LPS alone.
Conclusions: Cytokine secretion data from HSC and expression data from hepatocytes exposed to HSC supernatant show a specific and intriguing role for HSC during the orchestration of the acute phase response following endotoxemia. Besides being amplifiers of macrophage-induced cytokine signalling, these cells produce an independent secretory profile with an impact on hepatocytes that is different from direct effects induced by IL6 or LPS only.

P0429
DELETION OF TIMP-1 (EXON 3) DOES NOT AFFECT HEPATIC FIBROGENESIS AND CARCINOGENESIS
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Background and Aims: TIMP-1 is induced during hepatic fibrogenesis and considered to promote fibrosis in the injured liver by inhibition of matrix metalloproteases (MMP) and degradation of extracellular matrix. Moreover, TIMP-1 displays anti-apoptotic properties and has been shown to be upregulated in patients with hepatocellular carcinoma (HCC). Therefore, TIMP-1 could be a functional link between fibrosis and carcinogenesis in the liver.
Aim: To characterize the role of Timp-1 in hepatic fibrogenesis and carcinogenesis.

Methods: Hepatic fibrosis was induced in male wild-type (wt) and TIMP-1-knockout (TIMP-1$^{-/-}$) mice by ligation of the common bile duct (BDL) or administration of CCl_4. HCC formation was induced by administration of either diethylnitrosamine (DEN) alone or in combination with CCl_4 treatment. Liver fibrosis was assessed by sirius red staining. Expression of fibrosis-related genes was quantified by real-time PCR. Liver injury was determined by ALT level measurement. Tumorload was quantified by counting the number of visible tumors and measuring the size of the largest tumor with a caliper.
Results: Both, wt and TIMP-1$^{-/-}$ mice developed hepatic fibrosis after BDL and CCl_4 as evaluated by sirius red staining. We could not detect the anticipated attenuation of liver fibrosis in TIMP-1$^{-/-}$ mice in either model (mean area of red BDL: wt 3.5% vs. TIMP-1$^{-/-}$ 3.0%, ns; CCl_4: wt 1.0% vs. TIMP-1$^{-/-}$ 0.9%, ns). Quantification of fibrosis-related genes showed similar expression of alpha-SMA, TGF-beta, Col1a1, MMP2, MMP9 mRNA. DEN administration led to formation of HCCs in both wt and Timp-1$^{-/-}$ mice at the age of nine months. Combined DEN/CCl_4 administration resulted in tumor formation after six months. In neither model significant differences in tumor number or size could be detected bewteen wt and Timp-1$^{-/-}$ mice (number of tumors: DEN alone: 13.9 vs. 16.6, ns, DEN + CCl_4: 17 vs. 22, ns; tumor diameter: DEN alone: 6.6 mm vs. 6.5, ns; DEN + CCl_4: 5.8 mm vs. 4.9 mm, ns).
Conclusions: TIMP-1 (exon 3) is dispensable for the development of hepatic fibrogenesis and carcinogenesis in mice.

P0430
DELTA LIKE LIGAND 4 DRIVES LIVER DAMAGE THROUGH REGULATING CHEMOKINES

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Background and Aims: Mutation of Jagged1 or Notch2 causes Alagille syndrome, a disease characterized by defective development of intralobular bile ducts and liver injury but no overt fibrosis. To date, the role of individual Notch ligand in liver fibrogenesis remains unknown.
Methods: Immunohistochemical and immunofluorescent staining were used to investigate the potential association between Notch ligands and liver fibrosis in 113 patients with chronic HBV infection. Notch ligands, which were found to express in patient myofibroblasts, were then functionally examined in carbon tetrachloride (CCl_4)- and bile duct ligation (BDL)-induced mouse liver damage models and cultured hepatic stellate cells (HSC), Kupffer cells (KC) and hepatocytes.
Results: Among detected Notch ligands, only Delta like ligand 4 (Dll4) expression was detected in myofibroblasts and correlated with inflammatory grades and fibrotic stages in HBV patients. Administration of recombinant Dll4 protein (rDll4) remarkably ameliorated hepatocyte apoptosis, inhibited inflammatory cell infiltration, decreased expression of cytokines and chemokines, and improved fibrosis in mice with 4 weeks CCl_4 challenge. *In vitro*, incubation with rDll4 did not directly influence apoptosis of hepatocytes, HSC activation, collagen I production in HSCs, and lipopolysaccharide (LPS)-induced cytokine expression in KCs. However, rDll4 administration remarkably decreased LPS-induced chemokine (e.g. CCL2) expression in both KCs and HSCs. In contrast to CCl_4 model, administration of rDll4 induced hepatic massive necrosis in BDL-operated mice and caused them to death within one week. Compared to sham BDL mice, inflammatory cell infiltration in liver tissues was remarkably inhibited in rDll4-treated BDL mice. In cirrhotic liver tissues, immunohistochemistry analyses demonstrated a significantly reverse association between CCL2 and Dll4 immune positivity.
Conclusions: Dll4 drives liver damage via regulating chemokines. Decrease of chemokines with rDll4 treatment exerts a compelling anti-inflammatory/anti-fibrotic role in CCl_4-induced chronic liver damage, whereas rDll4-mediated chemokine inhibition induces massive hepatic necrosis in BDL mice via disrupting set up of inflammation.

P0431
IL-4/IL-13 EXACERBATE LIVER FIBROSIS PROGRESSION THROUGH ALTERNATIVELY ACTIVATED MACROPHAGES

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Background and Aims: Liver fibrosis progression and regression are modulated by cells of the immune system. T cells play roles in regulating macrophages by secreting cytokines such as IL-12, IL-4 and IL-13, inducing polarization into classically and alternatively activated macrophages (CAM and AAM), resp. The function of the AAM in liver is largely unexplored.
Methods: To investigate the function of IL-4Ra, IL-4 and IL-13 in liver fibrosis, systemic IL-4/IL-13 double KO mice and T-cell specific deletion of IL-4/IL-13 and IL-4Ra were generated. Liver fibrosis progression was modeled by administration of oral CCL4 in increasing dose for 6 weeks, and spontaneous (incomplete) regression by discontinuing CCL4 for another 2 weeks. Liver was harvested for Sirius Red staining, hydroxyproline quantification, immunohistochemistry and qPCR. Sera were collected for ALT measurement. Liver immune cells were analyzed by FACS.
Results: Collagen deposition was significantly reduced in systemic IL-4/IL-13 double KO mice. This was accompanied by 6-fold reduction of procollagen a1(I) and α-SMA transcripts, and a decreased ALT indicating suppressed liver injury. Since IL-4/IL-13 mediate immune-suppressive function, double KO mice also showed an increase in CD4 and CD8 T cells, monocytes and macrophages/Kupffer cells as assessed by FACS, in line with a (Th1/CAM) suppressive effect after CCL4 treatment. However, although IL-4/IL-13ΔCD4 mice showed a reduced procollagen a1(I) expression and IL-4RaΔCD4 mice showed a decrease of total Hyp compared to control mice, most of the other parameter of fibrosis showed no significant difference.
Conclusions: We demonstrate an important role of IL-4/IL-13 signaling in CCL4-induced fibrogenesis, since mice deficient in IL-4/IL-13 showed dramatic reduction of collagen deposition. It appears that Th2 T cells are less important promoters than AAM. Combined with our previous results in IL-4Ra deficient mice, this confirms AAM as central regulators of fibrogenesis during liver fibrosis progression.

P0432

NLRP3 INFLAMMASOME EXPRESSION IS REGULATED BY NUCLEAR FACTOR-KAPPAB (NF-κB) IN CULTURED HEPATOCYTES

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Background and Aims: The inflammasomes are cytoplasmic multiprotein complexes that are responsible for the activation of inflammatory reactions. They mediate the cleavage and activation of caspase-1 and IL-1β that in turn leads to a complex network of cellular reactions that at the end initiate local and systemic inflammation [1]. We have shown that the NACHT, LRR and PYD domains-containing protein 3 (NLRP3) is virtually absent in untreated cultured hepatocytes while stimulation with lipopolysaccharides (LPS) results in strong activation of NLRP3 expression in these cells [2]. In the present study we investigated the impact of NF-κB signalling on NLRP3 activation in primary hepatocytes.
Methods: Murine primary hepatocytes were isolated according to the collagenase method [3]. The NF-κB activation inhibitor QNZ and the adenoviral expression vector Ad5-CMV-IκB(S32A/S36A) expressing a hemagglutinin-tagged human superrepressor of NF-κB [4] were used to block NF-κB signalling. After LPS challenge, the expression of TNF-α, IL-1β and NLRP3 was analysed by qRT-PCR, Western blot and immunohistochemistry. The influence of NF-κB signalling on NLRP3 expression was further investigated in a novel murine hepatocytic cell system that can be Cre-dependently depleted for the NF-κB essential modulator (NEMO).
Results: We found that QNZ blocks LPS-induced expression of TNF-α, IL-1β and NLRP3 as demonstrated by qRT-PCR, Western blot and immunohistochemistry. Even more, the basal expression that was observed in uninfected control cells or in cells that were infected with a control virus (Ad5-CMV-Luc) was abrogated. Likewise, the superrepressor of NF-κB prevents expression of NLRP3. In cells that were depleted for NEMO (and Caspase 8), we could confirm that there is a close link of NF-κB activity and NLRP3 expression. As a consequence, the expression of Lipocalin 2 representing a biomarker of hepatic inflammation was blunted suggesting that NF-κB activity plays an important role in mediating inflammasome activity.
Conclusions: We conclude that NF-κB signalling is a necessary prerequisite for proper activation of the NLRP3 inflammasome in primary hepatocytes. Our data further suggests that targeting the NF-κB pathway will be potentially therapeutic useful to block hepatic inflammasome activity.

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P0433

SELECTIVE LXR ALPHA INTESTINAL ACTIVATION REDUCES HEPATIC INFLAMMATION AND FIBROSIS DURING THE DEVELOPMENT OF CHRONIC LIVER INJURY

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Background and Aims: Hepatic fibrosis represents the wound-healing response of the liver to chronic injury and it is characterized by increased and altered deposition of newly generated extracellular matrix. The pathogenesis of liver fibrosis is not fully understood, leading to a lack in effective therapies. Liver X receptors (LXR α/β) are important regulators of lipid metabolism. While in the liver LXRs regulate cholesterol and fatty acid metabolism, intestinal LXRα activation has been implicated in reverse cholesterol transport pathway leading to high levels of the antinflammatory HDL. AIMS: to evaluate the effect of the selective intestinal LXRα activation on the development of hepatic fibrosis associated to chronic liver injury.
Methods: Male FVB/N mice (8–10 weeks old) with intestinal constitutive LXRα activation (iVP16LXRα) and their control (iVP16) were treated with twice weekly i.p. injection of Carbon Tetrachloride (1 μl/gr body weight) for 2 months.
Results: Immunohystochemistry for the macrophage marker F4–80 indicated lowered infiltration in iVP16LXRα liver ($p < 0.05$). Gene expression of the pro-inflammatory cytokines IL-6, TNFα, and MCP-1, and of the transcription factor NF-Kb were reduced in iVP16LXRα CCL4 mice ($p < 0.05$, $p < 0.05$, $p < 0.005$ and $p < 0.005$ respectively). Collagen synthesis and deposition was significantly reduced in iVP16LXRα mice compared to iVP16 as determined by type I collagen and TGFβ mRNA expression (both $p < 0.005$) and by Sirius Red morphometry ($p < 0.0005$). To study the mechanisms associated to the antinflammatory effect of intestinal LXRα activation, reverse cholesterol transport was proved by measuring intestinal gene expression of Abca1, that promotes the efflux of cholesterol; indeed Abca1 expression was increased in iVP16LXα CCL4 mice ($p < 0.005$) in line with the elevation of plasmatic HDL concentration in iVP16LXRα CCL4 mice ($p < 0.005$). Furthermore, intestinal LXRα activation is not associated to hepatic de novo lipogenesis as shown by gene expression of SREBP1c and FAS, and decreased triglyceride ($p < 0.005$) and cholesterol content ($p < 0.05$).
Conclusions: Specific intestinal LXRα activation reduces liver injury by increasing the level of the antinflammatory HDL cholesterol, thus leading to decreased hepatic fibrosis. Selective intestinal activation of LXRα might be considered as a new therapeutic approach to reduce liver fibrosis avoiding the occurrence of hepatic steatosis as a side effects associated to systemic LXR induction.

P0434

JNK1-DEPENDENT ER STRESS CONTRIBUTES TO HEPATIC STELLATE CELL ACTIVATION

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Background and Aims: The ability of a cell to sense, respond to and circumvent stress is essential for maintaining homeostasis. The endoplasmic reticulum (ER) is the site of synthesis, folding and modification of proteins where unfolded proteins can accumulate in stress conditions. This accumulation initiates an adaptive response which consists of three main signaling pathways, i.e. IRE1, PERK and ATF6. ER stress plays a role in the liver damage and chronicity during alcohol abuse, infection with the hepatitis B and C virus, non-alcoholic steatohepatitis and can even promote hepatocarcinogenesis. In addition, IRE1 activates JNK which is a crucial mediator of liver fibrogenesis. Our aim was to study the role of ER stress during early hepatic stellate cell (HSC) activation and investigate a possible link between JNK and ER stress in primary mouse HSCs.
Methods: In vitro and in vivo activated HSCs were analyzed for ER stress.

Results: The ER stress markers, XBP1spliced, BiP and Chop, showed an early peak in mRNA expression already 10 h after seeding primary mouse HSCs on plastic culture dishes, followed by a decreased expression at 24 h. Up to 10 days, the expression levels did not differ from the 24 h time point. This increased ER stress could also be seen in freshly isolated HSCs from mice 10 h after 1 CCl_4 injection suggesting that ER stress is an early event of HSC activation also in vivo. HSCs cultured as 3D spheroids showed prevention of early ER stress and expression of activation markers was inhibited compared to HSCs plated on plastic. However, when primary mouse HSCs were plated on a soft substrate (0.48 kPa), expression of activation markers was reduced but ER stress was not prevented. In addition, treatment of HSCs with JNK inhibitors prevents the ER stress and reduces culture-induced activation of primary mouse HSC. This role for JNK was confirmed using JNK1 KO mice where decreased ER stress and activation were observed when isolated HSCs were plated. No effect on ER stress and activation was seen in HSCs from JNK2 KO mice.
Conclusions: ER stress induction is an early event during HSC activation in vitro and in vivo. Inhibition of ER stress by culturing cells in 3D spheroids inhibits HSC activation while cells seeded on soft substrates are less activated but ER stress is not affected. Finally, we show that this ER stress is JNK1 dependent. Together this strongly suggests that JNK1-dependent ER stress contributes to HSC activation, but is not sufficient to drive the activation process.

P0435
IDENTIFICATION OF MIR-192 AS A NOVEL KEY REGULATOR OF QUIESCENCE MAINTENANCE IN HUMAN HEPATIC STELLATE CELLS

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Background and Aims: To identify regulatory pathways maintaining hepatic stellate cell (HSC) in a quiescent (q) phenotype is of outmost importance to develop new therapeutic strategies for the treatment of liver fibrosis. The aim of the present study is to identify miRNAs regulating the maintenance of the quiescent phenotype.
Methods: qHSCs and *in vitro* activated HSCs were assessed for gene and miRNA profiling by Affymetrix HG-U219 and miRNA-Taqman® Array. Expression profiles were integrated using the miRComb-R package. Functional studies were performed in human and mouse cultured HSCs.
Results: MiRNA analysis identified miR-192 as a highly expressed miRNA in isolated human quiescent HSC and down regulated in *in vitro* activated counterparts (Fold Change=8, $p = 0.01$). The miR-192 expression level was confirmed in whole liver of healthy and cirrhotic patients. However, no differences in plasma levels of miR-192 were found between cirrhotic and healthy patients. Computational integrative analysis of mRNA-miRNA expression profiles identified 18 predicted target genes for miR-192. In silico functional analysis with Ingenuity pathway software showed that miR-192 target mRNAs were significantly enriched in HSC activation canonical pathway. Integrative analysis was validated by over-expressing miR-192 in human HSC (Lx2) showing a reduction in expression in both predicted targets and activation markers. Assessment of miR-192 expression in different isolated liver cell types from mouse liver demonstrated that HSC are the main cell source of miR-192. The dynamics of miR-192 expression was assessed by isolating HSC at different time points from two fibrotic mouse models (CCL4 and BDL) showing that down-regulation of miR-192 takes place early in the progression of fibrosis. Moreover, functional validation was performed in mouse HSC. MiR-192 overexpression resulted in a significant reduction of both cell proliferation and migration.
Conclusions: miR-192 is a quiescent HSC enriched miRNA targeting genes involved in HSCs activation process and its expression is rapidly reduced upon in vitro and in vivo activation. These results suggest that miR-192 could be an important regulator of the maintenance of HSC quiescent phenotype and a potential target to interfere HSC activation.

P0436
PENTRAXIN-3 INDUCES HEPATIC STELLATE CELL ACTIVATION AND ATTENUATES THE INFLAMMATORY RESPONSE DURING HEPATIC INJURY

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Background and Aims: Pentraxin-3 (PTX3) is an acute phase protein locally released in response to pro-inflammatory stimuli regulating the innate immune system. However, the role of PTX3 in the liver and its potential participation in tissue remodeling is still unknown. The aim of this study was to investigate the role of PTX3 as a modulator of inflammation in liver injury.
Methods: PTX3 gene expression was assessed by quantitative PCR in primary human hepatic stellate cells (HSC), liver tissue from patients with compensated alcoholic cirrhosis ($n = 6$), alcoholic hepatitis (AH) ($n = 15$), control individuals ($n = 6$) and in experimental mouse models. PTX3 serum levels were determined by ELISA and tissue expression by inmunohistochemistry. Interference RNA and recombinant PTX3 were used in cultured cells and liver tissue slices from healthy and chronic CCl_4 treated mice.
Results: PTX3 hepatic gene expression was up-regulated in AH (FC=35; $p < 0.05$) and cirrhotic (FC= 6, $p < 0.05$) patients compared to healthy controls. PTX3 serum levels were increased in cirrhotic patients ($n = 60$) compared with healthy controls and serum levels were higher in patients with Child B and C compared to Child A ($p < 0.05$). Moreover, PTX3 expression was up-regulated in experimental models of chronic CCl_4 treatment and acute-on-chronic liver injury (chronic CCl_4 and LPS) ($p < 0.01$). Assessment of FACS-sorted mouse hepatic cell populations showed that neutrophils were the main PTX3 producing cell type in healthy liver, whereas macrophages and mainly HSC were the main cells expressing PTX3 in liver injury. Cultured activated HSCs and macrophages expressed PTX3 that was up-regulated upon stimulation with pro-inflammatory agents (TNFα, IL1β, LPS). While knock-down of PTX3 in HSCs (Lx2) induced a reduction of activation markers (α-SMA, COL1A1, LOX) ($p < 0.01$), stimulation with recombinant PTX3 induced activation of human primary HSC. Next, we evaluated the effect of PTX3 on healthy and fibrotic liver tissue slices showing that PTX3 attenuated the inflammatory response induced by LPS, reducing the expression of TNFα, MCP-1, CCL20, IL-6, RANTES.
Conclusions: PTX3 hepatic expression is increased in cirrhotic and HA patients and it is associated with disease severity. PTX3 is produced by HSCs and macrophages in liver injury, which may

induce HSC activation and modulate tissue inflammation. These results suggest that PTX3 is a novel marker of liver injury and might exert a role in promoting tissue remodeling and attenuating inflammation.

P0437

REACTIVE GAMMA-KETOALDEHYDES AS NOVEL ACTIVATORS OF HEPATIC STELLATE CELLS IN VITRO

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Background and Aims: Reactive lipid aldehydes formed during lipid oxidation such as 4-hydroxynonenal (4-HNE), are key activators of hepatic stellate cells (HSCs) to a pro-fibrogenic phenotype. γ-Ketoaldehydes (γ-KAs) are a family of acyclic highly reactive aldehydes, comprising the levuglandins, and their isomers (isolevuglandins), which are formed during oxidation of arachidonic acid, or as a by-product of the cyclooxygenase pathway. γ-KAs are highly reactive and form protein adducts and cross-links at a rate >100-fold compared to 4-HNE. Increased circulating concentrations of proteins cross-linked to γ-KAs are present in patients with alcoholic liver disease. Since the contribution of γ-KAs to liver injury has not been studied, we investigated whether the γ-KA levuglandin E_2 (LGE_2) can induce activation of HSCs.
Methods: Culture-activated, serum-starved primary human HSCs were exposed to LGE_2 (0.5pM– 5μM) for up to 48 hours. Endpoints measured included proliferation, cytotoxicity [lactate dehydrogenase (LDH) and MTS assays], RNA and protein expression, and collagen secretion in conditioned medium.
Results: Within hours, exposure to 5μM LGE_2 promoted severe cytotoxicity and apoptosis, indicated by LDH leakage, reduced MTS, increased PARP cleavage, JNK phosphorylation, and increased levels of the pro-apoptotic transcription factor CHOP. At lower, non-cytotoxic doses (50pM–500nM) LGE_2 promoted HSC activation as indicated by increased expression of a-SMA and vimentin, and sustained activation of selective signaling pathways (p42/44 ERK1/2 and JNK/SAPK), but had no effect on DNA synthesis or collagen production. Further, HSCs exposed to LGE_2 displayed a marked increase in mRNA expression for various cyto/chemokines including interleukin-8 (IL-8), CCL2/MCP-1, IL-6, and IL-1β. This was accompanied by increased secretion of bioactive IL-1β/IL-18, as measured by HEK-Blue reporter cells. The JNK inhibitor SP600125 prevented activation and expression of mRNA of these cyto/chemokines by LGE_2 but inhibitors of activation of NFkB (PDTC) or p38MAPK (SB203580) had no effect. Lastly, and of possible relevance for their mechanism of action, γ-KAs exposure also increased indices of endoplasmic reticulum stress, such as GRP78, ATF4, and IRE1 phosphorylation.
Conclusions: γ-KAs represent a newly identified class of activators of HSCs *in vitro*, which are biologically active at concentrations as low as 50pM, and are particularly effective at promoting a pro-inflammatory response.

P0439

HUMAN 3D HEPATIC CO-CULTURE MODEL FOR IN VITRO DRUG-INDUCED FIBROSIS TESTING

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Background and Aims: In Europe, liver cirrhosis accounts for around 170,000 deaths a year according to WHO. The lack of clinically efficient treatment for fibrosis, together with the fact that the pro-fibrotic profile of a drug is often detected only at later stages of the preclinical phase, highlights the need for better human models to study fibrosis. The aim of our work is to develop an *in vitro* model where drug-induced liver fibrosis can be mimicked. An efficient model as such, would not only lead to an early retraction during drug development of pro-fibrotic substances but also to develop treatments for liver fibrosis. Liver fibrosis is the result of scar tissue formation as a consequence of long term ECM deposition by activated hepatic stellate cells (HSCs). *In vitro*, HSCs can be activated directly but in the majority of the cases *in vivo* it is a response to hepatocyte injury.
Methods: We developed a 3D human co-culture model of primary HSCs and hepatocyte-like cells (HepaRG) with the aim of screening both pro- and anti-fibrotic compounds.
Results: Here we present the optimized 3D HepaRG/HSC co-culture conditions that allow the maintenance of non-activated HSCs for at least 21 days based on the gene expression of HSC markers Col1a1, Col3a1 and Loxl2. In parallel, no alteration of hepatocyte functionality including CYP induction, albumin secretion and hepatocyte-specific gene expression is observed as compared to 3D HepaRG monocultures. However, TGFb can still directly induce activation of the human HSCs while LPS induces a survival response (IL6, MMP1, CCL2, TNFa, ILb). When exposed to known hepatotoxicants, HSC activation is observed by the increased mRNA expression of HSC activation markers and collagen protein accumulation and secretion. This effect is enhanced when the co-cultures are exposed to an inflammatory cytokine cocktail reflecting inflammation-induced fibrogenesis.
Conclusions: The 3D hepatic co-cultures of primary HSCs and HepaRGs are an attractive tool to predict the (anti-) fibrotic profile of a compound *in vitro*. This model constitutes a big step forward from the regular 2D HSC and 2D/3D HepaRG mono-cultures.

P0440

ANTIFIBROTIC EFFICACY OF TGF-BETA RECEPTOR ANTAGONIST IN PRECISION-CUT HUMAN AND RAT LIVER SLICES

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Background and Aims: Transforming growth factor beta (TGFβ) is recognized as a significant mediator in liver fibrogenesis, and inhibition of its signaling pathway is a potential target of antifibrotic agents. This study aimed to elucidate the antifibrotic efficacy of TGFβ receptor antagonist, LY2109761, and the involvement of the TGFβ signaling pathway in the early onset of fibrosis in precision-cut human and rat liver slices.
Methods: Precision-cut human and rat liver slices were incubated with LY2109761 for 48 and 72 hours. Viability of liver slices was assessed by the ATP content. The concentrations of LY2109761 used were non-toxic and in line with other in vitro models. Phosphorylated Smad2 and total Smad2, downstream signaling proteins of TGFβ pathway, were assessed by western blot. Gene expression of the fibrosis markers: Pro-collagen1A1 (PCOL1A1), α-Smooth Muscle Actin (αSMA), and Heat Shock Protein 47 (HSP47), were assessed by real-time qPCR.
Results: Activation of TGFβ signaling was observed during incubation with an increase in both phosphorylated Smad2 (36.9 and 2.4 fold) and total Smad2 (8.4 and 24 fold), in human and rat liver slices respectively. LY2109761 significantly inhibited phosphorylation of Smad2 up to 82±3% in human and up to 79±9% in rat liver slices. In contrast, total Smad2 expression was not affected. Gene expression of the fibrosis markers increased during incubation: PCOL1A1; 5.1 and 17.2 fold, αSMA; 1.1 and 2.3 fold, and HSP47; 1.6 and 5.3 fold, in human and rat liver slices respectively.

Following treatment with LY2109761, PCOL1A1 gene expression was significantly dose-dependently inhibited up to 96±2% in human and up to 95±2% in rat liver slices. αSMA gene expression was significantly inhibited up to 82±5% in rat liver slices. In contrast, HSP47 gene expression was significantly inhibited up to 66±6% in human liver slices.
Conclusions: TGFβ receptor antagonist shows antifibrotic efficacy in precision-cut liver slices. It significantly inhibits TGFβ signaling via the inhibition of Smad2 phosphorylation. Furthermore, inhibition of TGFβ signaling diminishes PCOL1A1 gene expression in both species, suggesting that PCOL1A1 is directly regulated by this signaling pathway. Species differences might exist in the regulation of αSMA and HSP47 by TGFβ. Furthermore, the results of this study reveal the involvement of TGFβ signaling pathway in the early onset of fibrosis in precision-cut human and rat liver slices.

P0441
TACKLING FIBROSIS USING TGF-β2 TARGETED AONS IN VIVO

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Background and Aims: TGFβ2 has not been investigated thoroughly in CLD progression and HCC. After providing evidence that TGFβ2 plays a putative role in fibrogenesis, we now aim to selectively target TGFβ2 expression using antisense oligonucleotides (AONs) for attenuation or blockage of human liver disease progression.
Methods: Three CLD mouse models (CCl_4, BDL and $Mdr2^{-/-}$) were investigated representing different types of CLD background. TGFβ2 and TGFβ1 expression were compared by qRT-PCR. Human TGFβ2 mRNA, was specifically targeted using a 18mer AON in the hepatic cell line LX2. In vivo, we selectively inhibited TGFβ2 using AONs. For induction of chronic liver damage, mice were injected i.p. with CCl_4 twice/week for 4 weeks. After 2 weeks, subcutaneous AON application started in parallel twice per week. In the MDR2-KO model, the AON was administered for 4 weeks. The effect and efficacy of AON treatment was evaluated by tissue morphology and on protein and mRNA level. Typical fibrotic markers are currently investigated.
Results: In the CCl_4 liver injury and regeneration model, relative expression dynamics were similar for both TGFβ isoforms. HSC activation and collagen expression were accompanied with transient increases of TGFβ2 expression. Samples of MDR2-KO mice showed frequent TGFβ2 upregulation within 3 to 15 month as compared to wt. Accordingly, an elevation of TGFβ2 expression was determined 14 days after bile duct ligation (BDL). Interestingly, while TGFβ2 was strongly induced after 14 days, there was only slight induction of TGFβ1 expression at this time. In human HCC specimen with different etiologies, TGFβ2 was found to be prevalently expressed in the stroma. Immunohistological assessment of in vivo AON distribution after 24 h and 5 days revealed strongest signals in liver and kidneys. CCl_4 injured AON-treated animals showed reduced TGFβ2 mRNA expression levels. Hydroxyproline content, a surrogate marker of fibrosis, was significantly reduced in both injury models upon AON-treatment as compared to controls. Sirius Red staining also morphologically revealed a significant reduction (~34%) of collagen deposition in AON-treated mice.
Conclusions: Taken together, our results suggest a role of TGFβ2 in the process of CLD. We further conclude that in vivo application of TGFβ2 directed AON to CLD mouse models could contribute to fibrogenesis attenuation. Further studies are currently performed to determine mechanistic details of AON effects and define specifications of a potential AON based treatment of CLD.

P0442
UDCA-LPE MODULATES DIFFERENT SIGNALING PATHWAYS INVOLVED IN HEPATIC FIBROGENESIS

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Background and Aims: Liver fibrosis with deposition and remodeling of extracellular matrix (ECM) is regulated by different signaling pathways. Ursodeoxycholyl Lysophosphatidylethanolamide (UDCA-LPE) is a synthetic bile acid-phospholipid conjugate with hepatoprotective and anti-fibrotic functions *in vitro* and *in vivo*. In this study we aim to elucidate signaling pathways, which mediate anti-fibrogenic action of UDCA-LPE.
Results: At membrane level ECM interacts with Integrin receptors, which promotes pro-fibrogenic signaling. After phosphorylation in response to Integrin engagement, FAK and SRC kinase mediate activation of myofibroblasts. After UDCA-LPE binding to Integrin β1, FAK is shortly activated by phosphorylation at Tyr 397 in CL48 cells. However, the binding of UDCALPE pulls Integrin β1 out from lipid raft, and consequently Integrin β1 loses the colocalization with SRC and FAK, which inhibits phosphorylation of FAK (Tyr 925 and Tyr 576/577) and SRC (Tyr416). The second messenger cAMP and cAMP dependent Protein Kinase A (PKA) can also suppress ECM production by inhibiting hepatic stellate cells differentiation. PKA is activated in 1 minute after UDCA-LPE treatment. Phosphorylation of ERK, B-RAF and C-RAF by UDCA-LPE was also investigated by western blot. UDCA-LPE induces pERK (Thr 202/Tyr 204). ERK is phosphorylated by MEK, which is mediated by B-RAF and C-RAF in CL48 cells. UDCA-LPE induces pB-RAF (Ser 445) and pC-RAF (Ser 338). We next investigated the crosstalk between Integrin, ERK and PKA signaling pathways. After Integrin-blocking Peptide RGD or FAK autophosphorylation inhibitor Y15 treatment, UDCA-LPE mediated phosphorylation of ERK is reduced and activation of C-RAF by UDCA-LPE is inhibited. These results suggest that activation of C-RAF and ERK by UDCA-LPE is dependent on Integrin-FAK signaling pathway. EGFR inhibitor AG1478 treatment also indicates, activation of C-RAF and ERK by UDCA-LPE is EGFR dependent. PKA inhibitor Rp-cAMP reduces pERK and inhibits B-RAF phosphorylation, suggesting activation of B-RAF and ERK is PKA dependent.
Conclusions: UDCA-LPE disturbs pro-fibrogenic integrin signaling by inhibiting FAK and SRC activity. Integrin and FAK are identified as an upstream signaling of activation of the EGFR/C-RAF/ERK pathway. UDCA-LPE further activates PKA, which subsequently induces B-RAF/ERK signaling. By inhibiting pro-fibrogenic pathways with concomitant activation of pro-proliferative ERK signaling, UDCA-LPE may improve hepatofibrogenesis and accelerate liver regeneration.

P0443
HYPERAMMONEMIA ACTIVATES HUMAN HEPATIC STELLATE CELLS AND IS A TARGET FOR TREATMENT OF PORTAL HYPERTENSION

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Background and Aims: Ammonia induces astrocyte swelling and brain inflammation in cirrhotic animals. Ammonia lowering with Ornithine Phenylacetate (OP) in Bile Duct Ligated (BDL) rats reduces brain water, increases brain eNOS activity and reduces coma stage. Given the genotypic and phenotypic homology between astrocytes and hepatic stellate cells (HSC) [HSCs contain glutamine synthetase (GS) and astrocytes express HSC markers], this study tested the hypothesis that ammonia (i) modulates HSC activation and (ii) the

use of ammonia lowering agent, OP, lowers portal pressure in BDL rats, through reduced HSC activation.
Methods: *In vitro:* Primary humanHSC (hHSC) were cultured. Effects of NH_4Cl challenge (0.1–10mM over 24–72 hrs) on hHSC to proliferation (BrdU), metabolic activity (MTS assay), viability (Neutral-Red), ultrastructural changes (EM) and gene/protein expression were studied. To test recovery, ammonia treated cells were replenished with glutamine and in separate experiments, pre-treated with L-methionine-sulfoximine (MSO-GS inhibitor) to determine the importance of GS. *In vivo*: 28-day BDL rats were treated with saline or OP for 5 days and portal pressure measured at termination and tissues were harvested for studies.
Results: *In vitro:* Hyperammonemia in primary hHSC induced time-dependent decreases in proliferation and metabolic activity, whilst inducing cell swelling and a myofibroblast-like phenotype even at 50–100umol/L. Ultrastructurally, ammonia-treated hHSC caused a dose-dependent ER enlargement and this was reversible by replenishing the culture with glutamine. NH_3 inhibition of hHSC proliferation was dependent on GS activity as MSO with hyperammonemia induced cell detachment and prevention of recovery suggesting that glutamine is important for hHSC survival. *In vivo:* BDL rats, with hyperammonemia had increased hepatic expression of pro-fibrogenic hHSC-related genes (α-SMA, PDGFb-R, Myosin IIA/IIB and Coll1), low eNOS activity and DDAH-1 and high portal pressure (14.4 ± 0.8 mmHg), all of which were corrected by OP treatment (PP: 11.1 ± 0.3 mmHg, $P < 0.01$).
Conclusions: These novel data suggest that hyperammonemia modifies hHSC's and imparts a swollen myofibroblast phenotype, which is reversible upon ammonia reduction. *In vivo* ammonia lowering decreases pro-fibrogenic and activated HSC gene and protein expression and lowers portal pressure, highlighting ammonia as a target for portal hypertension therapy and the key role of HSC in this process.

P0444
THE HIPPO PATHWAY EFFECTOR YAP CONTROLS MOUSE HEPATIC STELLATE CELL ACTIVATION

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Background and Aims: Hepatic stellate cell activation is a wound-healing response to liver injury. However, continued activation of stellate cells during chronic liver damage causes excessive matrix deposition and the formation of pathological scar tissue leading to fibrosis and ultimately cirrhosis. The importance of sustained stellate cell activation for this pathological process is well recognized, and several signaling pathways that can promote stellate cell activation have been identified, such as the TGFβ-, PDGF-, and LPS-dependent pathways. However, the mechanisms that trigger and drive the early steps in activation are not well understood.
Methods: Primary HSCs are isolated from healthy and fibrotic BalbC mice. Microarray analysis on in vitro and in vivo activated HSCs (unseeded), identified significant changes in genes related to the Hippo pathway. 2D and 3D spheroid cultures are used to investigate the contribution of the hippo-effector protein YAP.
Results: We identified the Hippo pathway and its effector YAP as a key pathway that controls stellate cell activation. YAP is a transcriptional co-activator and we found that it drives the earliest changes in gene expression during stellate cell activation. Activation of stellate cells *in vivo* by CCl_4 administration or activation *in vitro*, caused rapid activation of YAP as revealed by its nuclear translocation and induction of target genes. Importantly, knock-down of YAP expression or pharmacological inhibition of YAP prevented hepatic stellate cell activation *in vitro*. Moreover, *in vivo* inhibition of YAP activity with a pharmacological inhibitor impeded fibrogenesis in mice.
Conclusions: YAP activation is thus a critical driver of hepatic stellate cell activation and inhibition of YAP presents a novel approach for the treatment of liver fibrosis.

P0445
OBETICHOLIC ACID, AN FXR AGONIST, REDUCES HEPATIC FIBROSIS IN A RAT MODEL OF TOXIC CIRRHOSIS

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Background and Aims: Hepatic stellate cells (HSC) drive hepatic fibrosis following chronic liver injury. Although controversial, the bile acid-responsive farnesoid-X receptor (FXR) might antagonize hepatic fibrogenesis via inhibition of HSC activation. We aimed to investigate both the preventive and therapeutic effects of the FXR agonist obeticholic acid (INT-747) on fibrosis in a rat model of toxic cirrhosis.
Methods: Cirrhosis was induced by thioacetamide (TAA) intoxication for 18 weeks. INT-747 was given during the last 4 weeks of (prophylactic) or for 4 weeks after ending (therapeutic) intoxication. Vehicle-treated rats served as controls. At sacrifice, degree of fibrosis (image analysis, hydroxyproline), hemodynamic (liver perfusion) and biochemical parameters were assessed. HSC activation, proliferation, apoptosis, hepatic nuclear-factor kappa B (NF-κB) activation, pro-inflammatory and pro-fibrotic cytokines were determined (RT-PCR, western blot). The effect of INT-747 was further evaluated on these latter parameters on isolated HSC, Kupffer cells, hepatocytes and liver sinusoidal endothelial cells.
Results: INT-747 significantly decreased fibrogenesis during TAA-administration and reversed fibrosis in established cirrhosis. As a consequence, portal pressure decreased through reduced total intrahepatic vascular resistance. These beneficial effect related to HSC de-activation by decreased expression of pro-fibrotic (transforming growth-factor β, connective tissue growth factor, platelet-derived growth factor β-receptor, tissue inhibitor of metallopeptidase-1) and pro-inflammatory cytokines (e.g. monocyte chemo-attractant protein-1) via down-regulated NF-κB. INT-747 also affected hepatic markers of cell turn-over. In vitro, a direct effect of INT-747 on HSC was excluded.
Conclusions: FXR agonist INT-747 reverses fibrosis in toxic cirrhotic rats by decreased HSC activation, indirectly via decreased hepatic inflammation.

P0446

INHIBITORY EFFECT OF DIETARY CAPSAICIN ON LIVER FIBROSIS IN MICE

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Background and Aims: The activation of hepatic stellate cells (HSCs) is the main event during liver fibrosis development. Capsaicin, the active pungent compound of chili peppers, is widely consumed as food additive. In the liver, it can modulate the activation, proliferation and migration of HSCs in vitro. The aim of this study is to evaluate the inhibitory effects of dietary capsaicin on HSCs related to cholestatic and hepatotoxic-induced liver fibrosis in mice and its underlying mechanism of action.
Methods: In vivo: male Balb/c mice (n = 5 per group) received dietary capsaicin (0.01% mixed in the chow) after 3 days of bile duct ligation (BDL) or before and during chronic carbon tetrachloride (CCl_4) treatment. Liver tissue sections were investigated by histology, immunohistochemistry and real-time PCR. HSCs were FACS-isolated and activation markers were analyzed by real-time PCR. In vitro: freshly isolated HSCs were electroporated with DsRed-GFP-LC3B plasmid, cultured and treated with 100 μM capsaicin for 7 days and the autophagic flux quantified using ImageJ.
Results: Dietary capsaicin partially improved liver damage in BDL mice and inhibited further progression of the injury. The beneficial effects were accompanied by fewer necrotic areas, a decrease in collagen deposition and downregulation of profibrogenic markers in isolated HSCs. In the CCl_4 model, dietary capsaicin prevented livers from injury development and inhibited the upregulation of activation markers. However, capsaicin could not attenuate the CCl_4-induced fibrosis when it was already established. Furthermore, capsaicin-treatment reduced autophagic flux.
Conclusions: Dietary capsaicin decreases the severity of liver injury in murine BDL and hinders fibrogenesis in the BDL and CCl_4-induced liver fibrosis models. This anti-fibrotic potential of capsaicin could partly be due to its inhibitory effect on autophagic flux. Collectively, these results support that daily dietary consumption of capsaicin has beneficial effects on liver injury, especially on HSC activation.

P0447

CHANGES IN LIVER EXTRACELLULAR MATRIX DURING LIVER INJURY REGULATE HEPATIC PROGENITOR CELL PROLIFERATION AND DIFFERENTIATION

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Background and Aims: The extracellular matrix (ECM) is composed of proteins and other macromolecules that forms a network between cells. ECM is a dynamic structure and its remodelling is tightly controlled in normal liver homeostasis. Life-threatening pathological conditions such as liver cirrhosis and liver cancer arise when ECM remodelling becomes excessive or uncontrolled. Liver fibrosis is characterised by major changes in the liver ECM composition with the steepest increase in type I collagen deposition. In addition to collagens, laminins and fibronectin, the ECM is composed of other various smaller molecules that are tissue specifically disposed at the sites of injury. Our aim was to characterise the changes in the liver ECM components in an injured liver and to investigate how they influence liver regeneration.
Methods: We utilised perfusion-based chemical decellularisation of the mouse liver combined with quantitative analysis of the proteome to identify which ECM proteins are significantly upregulated or downregulated during acute liver injury. The expression profile of the differentially expressed proteins was characterised by immunohistochemistry and microscopy. The effect of the selected ECM proteins on hepatic progenitor cell proliferation and differentiation was evaluated in in vitro culture.
Results: We identified 4 structural and 4 regulatory ECM components that were upregulated during liver injury. 12 structural and 3 regulatory ECM components were found to be significantly downregulated. A strong increase in collagens type I, type V and fibronectin indicate that liver damage in acute mouse liver injury model is mainly characterised by fibrosis which is accompanied with the reduction of the basal membrane components and elastic fibers. Analysis of the *in vitro* culture of hepatic progenitor cells on selected substrates revealed that proteins upregulated during liver injury mainly supported cell proliferation but proteins that are reduced in damaged ECM support cell differentiation.
Conclusions: Changes in the liver ECM during liver damage regulate the regenerative response which is important for repairing the damage. Our findings indicate that acute liver injury causes a significant change in the balance of main structural liver ECM components and upregulation of several regulatory ECM proteins which together support the proliferation of hepatic progenitor cells and liver regeneration.

P0448

BENEFITS OF ISOLATING HSC USING FLUORESCENCE-BASED CELL SORTING

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Background and Aims: Hepatic stellate cells (HSC) are the main effector cells for liver fibrosis. Thus, isolation of highly pure primary HSC from healthy or diseased mouse liver is an evitable tool for functional studies on HSC biology and evaluating antifibrotic approaches *in vitro*. At present, most protocols for isolating primary murine HSC employ density gradient centrifugation only, however, this may result in contaminations with other cell types such as Kupffer cells (KC). We aimed at optimizing HSC isolation by adding an additional subsequent step of fluorescence-activated cell sorting (FACS) via a UV laser.
Methods: HSC were isolated from livers of healthy mice and mice subjected to experimental fibrosis. HSC isolation by iohexol-based density centrifugation was compared to a method with subsequent FACS-based sorting. We assessed cellular purity, viability, morphology, and functional properties like proliferation, migration, activation marker and collagen expression.
Results: FACS-augmented isolation resulted in a significantly increased purity of stellate cells (>99%) compared to iohexol-based density centrifugation alone (60–95%), primarily by excluding HSC-KC cell doublets. Importantly, this method is also applicable to young animals and mice with liver fibrosis. Viability and characteristics of HSC transdifferentiation (αSMA, collagen, desmin) *in vitro* were preserved upon FACS-based isolation. During the maturation of primary HSC in culture, we did not observe HSC proliferation by time lapse microscopy for several days. Strikingly, FACS-isolated, differentiated HSC showed very limited responses to lipopolysaccharide (LPS) stimulation.
Conclusions: Isolating HSC from mouse liver by adding an additional step of cell sorting significantly increased cell purity by removing contaminations from other cell populations especially KC, without affecting viability or differentiation.

P0449

NON STEROIDAL ANTI INFLAMMATORY DRUGS IN LIVER FIBROSIS

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Background and Aims: Liver fibrosis is an abnormal wound healing process resulting in extracellular matrix accumulation. If not treated, liver fibrosis leads to cirrhosis, which is a non-reversible stage of the disease. It is known liver fibrosis triggers the infiltration of inflammatory cells into the liver tissue and necrosis. Therefore, alteration of the inflammatory process in damaged liver may result in the regression of fibrosis. On the other hand, non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to prevent the inflammatory processes in various diseases. Clinically used NSAIDs, Aspirin, Ibuprofen, Naproxen and Flurbiprofen have been reported to be non-hepatotoxic. In this study we investigated the effects of these drugs and some novel NSAI compounds on the liver fibrosis.
Methods: Liver fibrosis model was generated by i.p. treatment of C57BL/6 male mice with CCl_4 twice a week for 4 weeks. The daily doses of drugs solved in simple syrup and administered by gavage for five days than mice were sacrificed. Non-fibrotic male mice used as negative controls whereas fibrotic mice used as positive control. α-SMA, Collagen levels in liver tissues and serum ALT/AST levels were evaluated. Collagen staining levels were quantified by the region based pixel intensity normalization algorithm using MATLAB. We further investigated the ongoing inflammation in liver by analyzing the expression of macrophage and T-cell markers.
Results: We demonstrated a obvious decrease in liver α-SMA and collagen levels in NSAID treated group compared to non-treated group. In addition, the apparent decrease in collagen staining upon NSAID treatment was further evaluated by the quantitative image analysis. The serum AST levels showed a decrease in all treated groups. The comparative analysis of the drugs indicated that Naproxen had the most significant effects whereas least decrease in α-SMA, Collagen levels were observed with Ibuprofen. Although Flurbiprofen had similar bioactivities on tissue in α-SMA, Collagen levels; less decrease in serum AST level was found. Overall, we confirmed that there is a supression in this inflammatory process.
Conclusions: The oral administrations of common NSAIDs to treat the liver fibrosis in mice, promote the regression of this disease as demonstrated by both quantitative microcopy and serum AST levels. These drugs mainly eliminated by renal excretion, therefore based on our results, we propose the repurposing of non-hepatotoxic NSAIDs for the treatment of liver fibrosis in clinics.

P0450

KCa3.1 CHANNELS ARE UPREGULATED IN HEPATOCYTES OF CIRRHOTIC PATIENTS

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Background and Aims: KCa3.1 is a calcium activated potassium channel, which regulates proliferation, migration and cell volume. The channel has been identified in circulating blood cells (erythrocytes, lymphocytes and macrophages), endothelial and epithelial cells in colon and airway. Its inhibition is suggested as target for therapy of lung and kidney fibrosis. Little is know about its role in liver fibrosis. Recently, we found that KCa3.1 deficient mice show augmented hepatic fibrosis upon liver injury. The present study investigated KCa3.1 channel expression in hepatic stellate cells (HSC), hepatocytes and Kupffer cells in vitro, as well as expression and cellular localization in human liver biopsies.
Methods: *Cellular studies:* In primary isolated hepatocytes, Kupffer cells and HSC we measured KCa3.1 gene expression by quantitative polymerase chain reaction. We incubated quiescent and culture-activated hepatic stellate cells with the specific KCa3.1 channel inhibitor TRAM-34 and the activator SKA-31 and evaluated effect on mRNA expression of collagen 1, smooth muscle actin and tumor growth factor β.
Human biopsies: liver biopsies from 54 patients representing normal, alcoholic cirrhosis, autoimmune disease or severe inflammation or fibrosis due to other aetiologies were included. Immunohistochemical stain was performed for KCa3.1 channel expression. Double fluorescent immune stain was performed to investigate KCa3.1/macrophage co-localization.
Results: KCa3.1 channel inhibition with TRAM-34 suppressed collagen production in HSC, but did not change the myofibroblastic phenotype. No changes were seen by channel activation by SKA-31 KCa3.1 mRNA was 300 and 100 times higher in macrophages and hepatocytes compared to HSC.
In normal human biopsies KCa3.1 channel expression was low or absent, but an abundant expression was seen in hepatocytes of cirrhotic livers. The channel was found only in a minor percentage of the macrophages.
Conclusions: Our study questions the potential anti-fibrotic effect of channel blockers, but demonstrates an upregulation of KCa3.1 channels in hepatocytes during cirrhosis, which might play a pathogenic role.

P0451

LASER CAPTURE MICRODISSECTION REVEALS CELL-SPECIFIC DNA METHYLATION SIGNATURES IN KEY FIBROSIS MODIFIER GENES IN CHRONIC LIVER DISEASE

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Background and Aims: Recent studies examining differential DNA methylation of key genes known to regulate fibrosis in human chronic liver disease have been at the whole tissue level; however, it is likely that DNA methylation patterns are cell-specific. Thus, our aim was to examine whether DNA methylation signatures of key fibrosis modifier genes vary according to cell type and acinar location in human chronic liver disease.
Methods: Sections from explanted liver from 6 patients transplanted for ALD were stained with H+E and subjected to laser capture microdissection (LCM); areas containing (i) principally hepatocytes or (ii) fibrotic bands containing myofibroblasts were selected. DNA methylation profiles of PDGFA and PPARA were determined using pyrosequencing.
Results: PDGFA was significantly hypermethylated in hepatocytes relative to fibrotic bands containing myofibroblasts (CpG 1: 38±4.3% vs 25±2.15%, $p < 0.03$; CpG 2: 16.9±1.87% vs 11.3±1.29%, $p < 0.02$). In contrast, PPARA methylation was increased in fibrotic areas compared to hepatocytes, although this did not reach significance (CpG:1 1.42±0.22% vs 0.84±0.14%).
Conclusions: Cell-specific DNA methylation signatures exist in key fibrosis modifier genes in chronic liver disease. These results show that investigation of DNA methylation patterns in human diseases on an epigenome-wide scale should be interpreted with caution if cell specificity is not accounted for.

P0452

CHANGES IN DNA METHYLATION AND HYDROXYMETHYLATION DURING HSC ACTIVATION

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Background and Aims: DNA methylation (5mC) is an epigenetic mark that is an established regulator of gene transcription with an important role in liver fibrosis. Currently, there is only very basic knowledge available as to how DNA methylation controls the phenotype of hepatic stellate cells (HSC), the key cell type responsible for onset and progression of liver fibrosis. Evidence has shown that presence of higher levels of DNA methylation around gene promoters correlate with low or no transcriptional activity. However, recently discovered 5-hydroxymethylcytosine (5hmC), generated by oxidation of 5mC through action of TET enzymes, is actually involved in gene activation and its patterns are often altered in human diseases. In this study we investigated the role of 5mC and 5hmC in liver fibrosis.
Methods: Levels of 5mC and 5hmC were assessed by slot blot in a range of animal liver fibrosis models and diseased human livers. Expression levels of TET enzymes and DNA methyltransferases (DNMTs) were measured by qRT-PCR and WB. Reduced representation bisulfide sequencing (RRBS) method was used to examine 5mC and 5hmC patterns in quiescent and in vivo activated rat HSC.
Results: Expression of TET enzymes and therefore global levels of 5hmC, are both downregulated in activated rat HSC and in diseased human livers. Moreover, DNMTs expression is changed in human and rat samples suggesting changed levels of 5mC. Using RRBS, we examined the exact genomic positions of changed methylation patterns in quiescent and in vivo activated rat HSC.
Conclusions: We found significant differences in TET and DNMT protein expression between quiescent and activated rat HSC as well as in human samples, which in turn cause alteration in the HSC DNA methylome and hydroxymethylome. Changes in DNA methylation during HSC activation may bring new insights into the molecular events underpinning fibrogenesis and may provide biomarkers for disease progression as well as potential new drug targets.

Liver immunology including viral hepatitis

P0453

HSF1 REGULATES INFLAMMASOME NLRP3-MEDIATED INNATE IMMUNITY VIA ACTIVATING BETA-CATENIN SIGNALING IN LIVER ISCHEMIA AND REPERFUSION INJURY

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Background and Aims: Heat shock transcription factor 1 (HSF1), a major regulator of heat shock gene transcription, might play important roles in the regulation of cell stress and disease state. In response to injurious agents, the inflammasome NLRP3 mediates caspase-1 activity to drive innate immune response. Although heat shock response has been implicated to differentially regulate immune response, little is known the functional role of macrophage HSF1 in the regulation of inflammasome NLRP3 activation during liver ischemia and reperfusion injury (IRI). This study was designed to explore the intracellular signaling networks regulated by HSF1 in NLRP3-mediated innate immune response triggered by IR stress in a mouse liver.
Methods: Myeloid specific HSF1 knockout ($HSF1^{M\text{-}KO}$) and HSF1 transgenic ($HSF1^{flox/flox}$) mice (n = 6/group) were subjected to 90 min partial liver warm ischemia followed by 6 h of reperfusion. In parallel *in vitro* study, bone marrow-derived macrophages (BMMs) from $HSF1^{M\text{-}KO}$ or $HSF1^{flox/flox}$ were transfected with HSF1-expressing vector (pBabe-HSF1) or beta-catenin siRNA/nonspecific siRNA (100 nM), and then incubated with LPS (100 ng/ml).
Results: Myeloid-specific HSF1 knockout (HSF1M-KO) increased the expression of NLRP3 and cleaved caspase-1 leading to exacerbating hepatocellular injury, as evidenced by increased sALT levels and hepatocellular necrosis/apoptosis. Unlike in HSF1flox/flox controls, HSF1M-KO increased macrophage/neutrophil trafficking and pro-inflammatory IL-1β/IL-18 levels in ischemic livers. Consistent with the in vivo results, LPS-stimulated BMMs from HSF1M-KO markedly increased NLRP3/caspase-1 activity and IL-1β production. In contrast, transfection of LPS-stimulated BMMs with pBabe-HSF1 depressed NLRP3/caspase-1 yet increased β-catenin phosphorylation. Furthermore, knockdown of β-catenin with siRNA treatment in HSF1-expressing cells readily activated NLRP3/caspase-1 and TLR4, which in turn enhanced NF-κB activation and increased expression of proinflammatory mediators.
Conclusions: This study demonstrates that myeloid specific HSF1 deletion triggers inflammasome NLRP3/caspase-1 activation in IR-induced inflammation. Induction of HSF1 promotes β-catenin signaling, which in turn negatively regulates NLRP3/caspase-1 activation. By documenting the crucial role of macrophage HSF1 in the regulation of NLRP3-mediated innate immune-driven local inflammatory responses, our findings provide the rationale for a novel therapeutic strategy against IRI in organ transplant recipients.

P0454

AN ADDITIONAL HEME OXYGENASE-1 KNOCKOUT INCREASES MATURATION OF DENDRITIC CELLS AND LIVER INFLAMMATION IN Mdr2 KNOCKOUT MICE

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Background and Aims: Deletion of the multi drug resistance protein 2 (Mdr2) in mice causes hepatic inflammation and fibrosis with progression to hepatocellular carcinoma (HCC) at about 12 month of age (Mdr2 knockout; Mdr2ko; FVB.129P2-$Abcb4^{tm1Bor}$). We have recently shown that induction of heme oxygenase-1 (HO-1) in Mdr2ko mice interferes with liver inflammation, fibrosis formation and proliferation. We established a double knockout mouse for Mdr2 and HO-1 (Mdr2/HO-1ko) to further investigate effects of HO-1 on chronic liver inflammation and its consequences.
Methods: Liver damage was monitored by alanine aminotranferase (ALT) levels. Liver hydroxyproline content was measured as a fibrosis marker. Spleen weight was measured as a marker of inflammation. Leukocyte infiltration and neoductuli formation was visualized by H&E staining of liver slices. Flow cytometry was used to analyze liver immune cell populations. Bone marrow derived dendritic cells (BM-DCs) were analyzed for cytokine production and expression of maturation markers. Female and male (F/M) FVB/N background control (wt), Mdr2ko, and Mdr2/HO-1ko mice were analyzed at the age of 12 weeks.
Results: ALT levels were significantly up-regulated in Mdr2ko (F/M), and male Mdr2/HO-1ko mice compared to wt, while female Mdr2/HO-1ko display even higher ALT levels. Liver hydroxyproline levels were significantly higher in Mdr2/HO-1ko mice (F/M) compared to Mdr2ko mice (F/M). Spleen weight was doubled in Mdr2ko (F/M) mice compared to wt, while Mdr2/HO-1ko (F/M) mice showed a 5 fold elevated spleen weight. In histological liver

stainings, Mdr2ko and Mdr2/HO-1ko mice (F/M) showed similar levels of leukocyte infiltration and neoductuli formation and were significantly up-regulated compared to wt mice. Flow cytometry data showed increased frequencies of T-cells and NKT-cells as well as $CD11c^+$ dendritic cells (DCs) in Mdr2/HO-1ko mice compared to Mdr2ko mice. In addition DCs showed increased surface expression of CD86. BM-DCs from Mdr2/HO-1ko showed a 5 fold increase in MHCII-, and CD86-expression as well as a 5 fold increase in IL-12 production even without LPS restimulation compared to Mdr2ko.
Conclusions: Mdr2/HO-1ko mice have a stronger fibrotic and inflammatory phenotype compared to the single Mdr2ko. The stronger inflammation can be explained by higher frequencies of $CD3^+$ T-cells, NKT-cells and mature DCs. High production of IL-12 in BM-DCs indicate a stronger Th1 response in Mdr2/HO-1ko mice compared to Mdr2ko.

P0455
IL-4Ra REGULATES LIVER FIBROSIS DIFFERENTLY DURING PROGRESSION AND REVERSAL PHASES BY MODULATING THE RATIO OF M1 VS M2 MACROPHAGES

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Background and Aims: In response to various stimuli, macrophages can be functionally divided into M1 (inflammatory) and M2 (anti-inflammatory) groups. Alteration of the M1-M2 ratio likely impacts liver fibrosis progression and reversal. IL-4Ra, which is activated by IL-4 and IL-13 has been linked to M2 polarization. However, the functional role of IL-4Ra in liver fibrosis progression and reversal remained unclear.
Methods: Balb/c or C57BL6 mice with systemic or conditional deletion of the IL-4Ra were treated with CCL4 via oral gavage for 6 weeks, to induce advanced liver fibrosis (parenchymal progression model). The reversal phase is determined 2 weeks after the last treatment. Mdr2 KO mice model were used as a bililary fibrosis model. The extent of liver fibrosis was determined by hydroxyproline measurement and Sirius Red morphometry. Fibrosis and inflammation related transcripts were determined by qPCR. Macrophage subsets were quantified by FACS analysis and IHC. For therapeutic intervention, control or IL-4Ra specific antisense oligonucleotides injected i.p. into fibrotic mice.
Results: IL-4Ra was prominently upregulated in Mdr2 KO mice with spontaneous biliary fibrosis, and less markedly in CCL4-treated mice. CCL4-treated IL-4Ra KO mice had 25% less fibrosis than their wildtype littermates. T and B cells, granulocytes, M2 macrophages were significantly decreased, however, the M1 macrophages population were increased. To specifically characterize the function of IL-4Ra in macrophages, IL-4RaΔLysM mice were generated and treated with 6 weeks of CCL4. These mice showed no difference in T and B cell numbers, but a significant reduction of CD11bloF4/80hiCD206+ M2 resident macrophages. This was accompanied by significantly attenuated fibrosis in the $IL\text{-}4Ra^{\Delta LysM}$ mice. Surprisingly, in wildtype mice M2 macrophages were remarkably increased during the resolution phase of CCl_4-induced fibrosis (up to week 2 after discontinuation of CCL4). Here, fibrosis resolution was significantly retarded in $IL\text{-}4Ra^{\Delta LysM}$ mice. In the therapeutic setting, a potent antisense oligonucleotide against IL-4Ra inhibited the progression but retarded the regression of fibrosis.
Conclusions: Our studies demonstrate that IL-4Ra in general and in macrophages in particular modulates fibrosis progression and reversal in discordant ways. Unexpectedly, M2 macrophages contribute to fibrogenesis but speed up fibrolysis. Modulation of IL-4Ra by antisense oligonucleotides should be an effective therapy to retard immune mediated fibrosis progression.

P0456
RE-EVALUATION OF HBV CLINICAL PHASES BY SYSTEM BIOLOGY IDENTIFIES UNAPPRECIATED ROLES FOR THE INNATE IMMUNE RESPONSE AND B CELLS

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Background and Aims: Host factors that identify distinct clinical phases of a chronic HBV infection – immune tolerant (IT), immune active (IA), inactive carrier (IC) and HBeAg negative (ENEG) hepatitis phases – are poorly defined. We performed a systems biology study to identify host markers that can differentiate between these disease phases.
Methods: Serum samples from untreated chronic HBV patients ($n = 71$) were used for multiplex cytokine measurements, quantitative HBsAg, HBeAg levels, HBV genotype and mutant analysis. Leukocytes were phenotyped using multicolour flowcytometry and whole blood transcriptome profiles were generated.
Results: HBV viral load, HBeAg and HBsAg levels ($P < 0.001$), but not leukocyte composition differed significantly between distinct phases. Serum MCP-1, IL-12p40, IP-10, MIP-1β levels were different between two or more clinical phases ($P < 0.05$). The lowest number of HBV precore mutations was found in IT patients ($P < 0.01$). Comparison of blood transcriptomes identified 64 differentially expressed genes between clinical phases. The gene signature distinguishing IA from IT and IC patients was predominantly composed of highly upregulated immunoglobulin encoding genes. Furthermore, gene-set enrichment analysis corroborated abundant expression of B cell function-related genes in IA patients, and pointed towards increased ISG transcript levels in IT patients compared to subsequent phases. Finally, NK cell activities were clustered in clinical phases with biochemical liver damage (IA and ENEG phases).
Conclusions: HBV clinical phases are characterised by distinct blood gene signatures. Innate interferon and B cell responses are highly active during the IT and IA phases, respectively. This suggests that the presumed immune tolerance in chronic HBV infections needs to be redefined.

P0457
GRAPHENE QUANTUM DOTS ATTENUATE CONCANAVALIN A-INDUCED HEPATITIS

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Background and Aims: Reactive oxygen species (ROS) production regulates the function and activity of liver infiltrated immune

cells, particularly T cells and macrophages. Graphene quantum dots (GQD) are grapheme sheets with lateral dimensions less than 100 nm. Laser ablation-generated GQD produce ROS upon irradiation with blue light, while in the absence of photoexcitation they display a potent ROS-quenching ability. Since effects of GQD on either normal or pathologic immune responses have not been explored so far, by using Concanavalin A (Con A) induced hepatitis, we aimed to investigate immunomodulatory and cytoprotective effects of GQD in liver pathology.

Methods: We investigated the effects of GQD (50 mg/kg i.v.) in Con A-induced hepatitis (Con A; 12 mg/kg i.v.) by using wild type BALB/c and ST2 deficient mice. We estimated liver injury and inflammation by liver enzyme tests, histology, cytokine production, intracellular staining of immune cells, by determining apoptosis and autophagy in the liver.

Results: GQD accumulated in liver and reduced Con A-mediated liver damage, as demonstrated by histopathological analysis and a decrease in liver lipid peroxidation and serum levels of liver transaminases. The cleavage of apoptotic markers caspase-3/PARP and mRNA levels of proapoptotic mediators Puma, Noxa, Bax, Bak1, Bim, Apaf1, and p21, as well as LC3-I conversion to autophagosome-associated LC3-II and expression of autophagy-related (Atg) genes Atg4b, Atg7, Atg12, and beclin-1, were attenuated by GQD, indicating a decrease in both apoptosis and autophagy in the liver. This was associated with the reduced liver infiltration of immune cells, particularly the T cells producing pro-inflammatory cytokine IFN-γ, and a decrease in IFN-γ serum levels. In the spleen of GQD-exposed mice, mRNA expression of IFN-γ and its transcription factor T-bet was reduced, while that of the IL-33 ligand ST2 was increased. The hepatoprotective effect of GQD was less pronounced in ST2-deficient mice, indicating that it might depend on ST2 upregulation. In vitro, GQD inhibited splenocyte IFN-γ production, reduced the activation of extracellular signal-regulated kinase in macrophage and T cell lines, inhibited macrophage production of the free radical nitric oxide, and reduced its cytotoxicity towards hepatocyte cell line HepG2.

Conclusions: GQD alleviate immune-mediated fulminant hepatitis by interfering with T cell and macrophage activation and possibly by exerting a direct hepatoprotective effect.

P0458
AGGRAVATION OF LIVER DAMAGE IN Ceacam1 (CARCINOEMBRYONIC ANTIGEN-RELATED CELL ADHESION MOLECULE 1)-DEFICIENT MICE IN IMMUNE MEDIATED LIVER INJURY

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Background and Aims: The T cell mitogenic plant lectin concanavalin A (ConA) induces acute immune-mediated liver injury, evident by elevation of plasma transaminases (ALT) and release of IFNgamma and TNFalpha. The cellular adhesion molecule CEACAM1 is expressed on epithelia, endothelia, and leukocytes. CEACAM1 has been originally identified as an intercellular, homophilic adhesion molecule on hepatocytes. The CEACAM1 isoform with a long cytoplasmic domain contains an ITIM that is pivotal in for the negative regulation of leukocyte activation, more specifically, CEACAM1-long suppresses the activity of NK-cells, T cells and myeloid cells. This negative regulation is important in modulation of innate immunity in both infection and sterile inflammation. Thus, elucidation of CEACAM1-dependent regulatory mechanisms in immune mediated liver injury might open novel therapeutic perspectives for human liver disorders.

Methods: *Ceacam1*$^{-/-}$ and wildtype (wt) were injected with a sublethal dose of ConA (5 mg/kg) or saline, respectively. After 24 hrs, ALT activity and expression of cytokines were determined by ELISA. Splenic and hepatic T cell populations were analysed by FACS. Tregs were adoptively transferred from wt or *Ceacam1*$^{-/-}$ donors into wt recipients.

Results: ConA-induced liver damage was exacerbated in *Ceacam1*$^{-/-}$ mice, evident by significant elevation of ALT and an exaggerated Th1-cytokine response. Further, CEACAM1-expression on CD4+ T cells, CD4^{+}Foxp3^{+} regulatory T cells (Treg), and CD8^{+} T cells was increased after ConA treatment. *Ceacam1*$^{-/-}$ mice displayed lower Treg frequency in both liver and spleen. A protective effect of *Ceacam1*$^{-/-}$ Tregs in ConA hepatitis opposed to CEACAM1-expressing Tregs was not observed.

Conclusions: Aggravation of liver damage in *Ceacam1*$^{-/-}$ mice suggests a CEACAM1-dependent regulation of immune-mediated liver disease. Our data indicate an immune modulatory function of CEACAM1 in the ConA-model by regulating CD4^{+} and CD8^{+} T cell abundance and polarization. Future studies will describe the functional role of CEACAM1 in regulation of Treg activity and a Th1-polarized immune response yielding liver protection.

P0459
NATURAL KILLER CELL PHENOTYPIC PROFILE IN HEPATOCELLULAR CARCINOMA (HCC) IS PREDICTIVE OF CLINICAL OUTCOME AFTER CURATIVE TREATMENT

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Background and Aims: Natural killer (NK) cells are known to be involved in the immunological control of liver cancer. Limited studies have addressed phenotypic and functional characteristics of NK cell response in HCC. We investigated NK cell phenotype association with clinical outcome after liver resection or percutaneous ablation.

Methods: 76 consecutive Caucasian HCC patients with HCV-related liver disease and 12 patients with HCV-related liver cirrhosis as controls were evaluated. Phenotypic analysis was carried out by staining for NKCD56, NKG2A, NKG2D, NKp30, NKp46, CD3ζ, perforin and granzyme B. Data analysis was performed by unsupervised hierarchical clustering of NK cell subsets frequency. Overall survival of the generated patient clusters was analyzed by log rank test.

Results: Our data indicate a significantly higher level of NKG2A ($p < 0.0001$), CD3ζ ($p = 0.0002$) and perforin ($p = 0.017$) expression in cirrhotics (controls) compared to HCC patient.
Hierarchical clustering of HCC patients identified three clusters, one of which was characterized by significantly lower overall survival ($p = 0.0002$). Comparison of different parameters among clusters revealed significantly lower level of CD3ζ ($p < 0.0001$), granzyme B ($p = 0.0003$) and perforin (<0.0001) expression in patients with worse survival that maintained higher level of NKG2A ($p = 0.018$), NKp30 ($p = 0.034$) and NKp46 ($p = 0.0002$) compared to patients with better outcome.

Conclusions: The analysis of NK cell phenotype suggests a cytotoxic defect in HCC patients compared to patients with liver cirrhosis. This defect could play a role in the clinical outcome after curative treatments for HCC. Altogether these results further support the relevant role played by NK cells in the immunopathogenesis of HCC, indicating a rational for immunotherapeutic strategies aimed at restoring NK-mediated anti-tumor activity.

P0460

CD3hiCD4- Vγ9/Vδ2 TCR+ GAMMA DELTA T CELLS WITH TH1 AND NK-LIKE PHENOTYPE ARE INDUCED IN PATIENTS WITH CHRONIC HEPATITIS B

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Background and Aims: Gamma delta (γδ) T cells are non-conventional innate-like cells that recognize ligands induced by stress or pathogens and have potential roles in host defense, tumor surveillance, and immune regulation. Based on preliminary detection of CD3hiCD4- T cells expressing γδ T cell receptor (TCR) in blood of patients with chronic hepatitis B (cHBV), we asked if γδ T cells are: (1) induced during chronic and acute infection; (2) distinct in phenotype and function; (3) associated with clinical and virological status of HBV infection.

Methods: 200 subjects with cHBV, 20 uninfected controls and 12 subjects with acute hepatitis B (aHBV) were recruited for immune analysis between February 2011 and December 2013 from 10 medical centers in North America. The frequency, phenotype, and function of CD3hiCD4- γδ T cells in peripheral blood lymphocytes were examined by multi-parameter cytometry in all subjects, with further evaluation of γ/δ TCR subtype, cytokine profile and Tbet expression in 24 cHBV (12 with flares, 12 without flares), 8 aHBV and 15 uninfected controls. Statistical analysis included the use of non-parametric tests, Chi-square or Fisher's Exact test as appropriate.

Results: We report that: (1) circulating CD3hiCD4- γδ T cells (mostly Vγ9+Vδ2+) are increased in frequency in cHBV compared to aHBV or control subjects (median 3.1% vs. 0.7% vs 1%, $p < 0.0001$); (2) CD3hiCD4- γδ T cells from cHBV subjects showed reduced CD28 and PD-1 expression with increased NK receptor expression (e.g. CD56, CD94, CD158a, NKG2A) compared to conventional CD3intCD4- T cells. Further analysis of Vγ9+Vδ2+ γδ T cells showed that they display a Th1 (but not Th17) phenotype with markedly increased IFNγ production (median 49% vs 14%, $p < 0.0001$) and Tbet expression (MFI 7592 vs 635, $p < 0.0001$) compared to conventional CD3int CD4- T cells; (3) While γδ T cell frequency did not associate with sALT or HBV DNA titers, γδ T cells from cHBV patients with sALT flares showed surprisingly lower IFNγ production than γδ T cells from stable cHBV patients (median 41% vs. 61%, $p = 0.0116$).

CHB (n = 200)	*CD3hiCD4-γδ T cells	Conventional CD3int CD4- T cells	p-value (Wilcoxon)
%CD28	49.4	75.7	<0.0001
%PD-1	12.6	19.5	<0.0001
%CTLA-4	6.3	6.1	0.8666
%CD56	21.6	7.4	<0.0001
%CD94	60.3	7.9	<0.0001
%CD158a	6.6	4.5	0.0003
%NKG2A	40.5	4.9	<0.0001
%NKG2D	90.2	86.1	0.0038

Conclusions: Chronic but not acute hepatitis B is associated with increased frequency of circulating γδ T cells with a highly Th1 phenotype. Analysis is ongoing to define the role of γδ T cells in HBV pathogenesis and outcome, given their reduced Th1 phenotype in cHBV patients with sALT flare.

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INCREASED LIVER GAMMA DELTA T CELLS ACCOMPANIED BY ENHANCED INNATE IMMUNE RESPONSES IN MOUSE MODEL OF ACUTE HEPATITIS B VIRUS (HBV) INFECTION BY HYDRODYNAMICS-BASED IN VIVO TRANSFECTION OF HBV-DNA

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Background and Aims: It is well known that the immune responses to HBV antigens and HBV replication during the acute phase of HBV infection might play a key role in viral clearance. γδT cell is one type of innate immune cell which exerts functions in the early phase of immune responses. Our previous studies showed abnormal features of γδT cell from CHB patients. Here, a further study was conducted to investigate the characteristics of liver γδT cells as well as other innate immune responses during acute HBV infection, and to explore their roles in acute HBV infection and viral clearance.

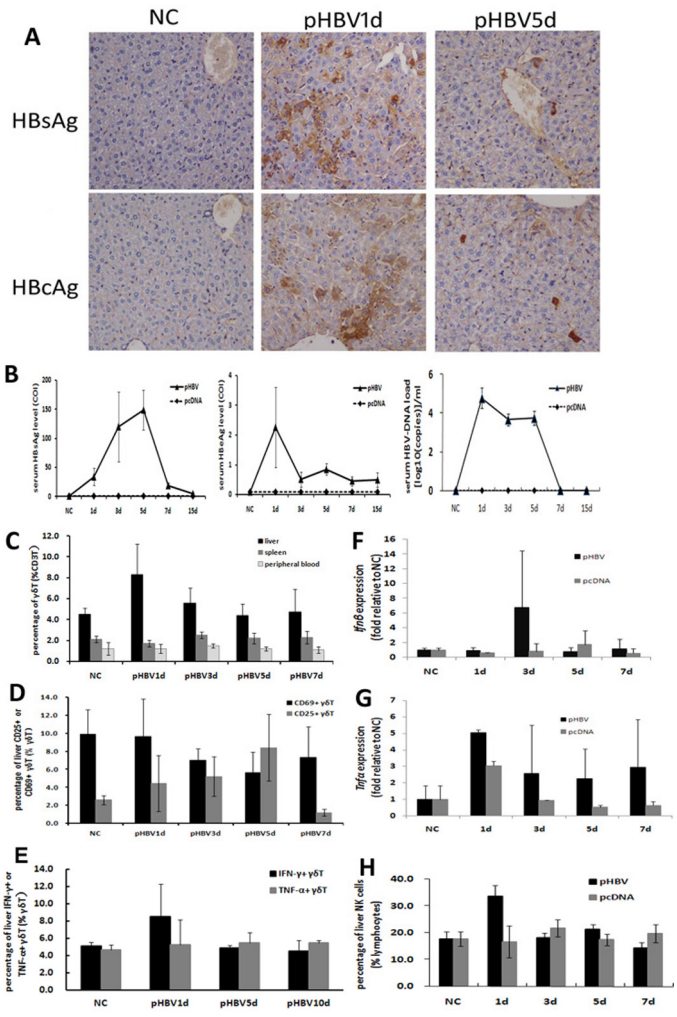

Figure: Changes in liver γδT cells and the innate immune responses in a murine acute HBV infection model. (A) HBsAg and HBcAg expression in liver tissue at 1d and 5d post injection with pHBV plasmid; (B) level of serum HBV antigen and HBV-DNA after pHBV plasmid injection; (C,D,E,H) percentage of liver γδT cells (C), CD25+ or CD69+ γδT cells (D), IFN-γ- or TNF-α-producing γδT cells (E), or NK cells after pHBV plasmid injection; (F,G) RNA expression of IFN-β and TNF-α in liver specimen after pHBV plasmid injection.

Methods: pcDNA-HBV1.3 (pHBV) plasmid containing HBV genome was injected into mice with hydrodynamics method. HBV antigenemia and serum HBV-DNA were measured with electrochemiluminescence and polymerase chain reaction (PCR) respectively. HBsAg and HBcAg expression in liver tissue were detected by immunohistochemical staining. Intrahepatic leukocytes were isolated, and flow cytometry was used to detect the percentage of γδT, CD25+ or CD69+ γδT, IFN-γ- or TNF-α-producing γδT cells, NK and T cells. Furthermore, total mRNA of liver tissue was prepared and real-time quantitive PCR was applied to detect gene expression involved in the innate immune responses, including TLR3, TLR7, TLR9, IRF3, IRF7, IFN-β, IFN-α, TNF-α and IL12a.
Results: At day 1 post injection (p.i), serum HBsAg, HBeAg and HBV-DNA were positive, and high percentage of HBsAg- or HBcAg-positive hepatocytes showed in liver specimen. Then liver HBsAg or HBcAg expression decreased sharply and showed negative after 7 days p.i. A murine acute HBV infection model was established. On day 1 p.i, the percentage of liver γδ T cells in pHBV group significantly increased (8.3±4.3%) compared to the normal mice (4.5±0.6%) and the pcDNA control group (3.8±1.6%). And liver γδ T also showed enhanced CD25 expression and IFN-γ production. At the same time, gene expression of IRF7, IFN-β or TNF-α in liver biopsies from pHBV group was significantly higher than pcDNA group. Furthermore, the percentage of liver NK cells sharply increased at 1 d p.i.
Conclusions: The percentage and functions of liver γδ T cells enhanced in the early phase of acute HBV infection. And these changes were correlated with hepatocyte HBV antigen expression and the boosted innate immune responses. Our results demonstrated that enhanced γδ T cells along with boosted innate immune responses might play important role in acute HBV infection and viral clearance.

P0462
CXCR6 MEDIATES RECRUITMENT OF ACTIVATED NATURAL KILLER CELLS IN CHRONIC LIVER DISEASE

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Background and Aims: The CXC-chemokine receptor CXCR6 is widely expressed on lymphocytes where expression is linked with a tissue-infiltrating effector phenotype. Previous work from our group has shown CXCL16, the ligand for CXCR6, promotes lymphocyte adhesion to cholangiocytes in inflamed human liver. In the present study we examined expression of CXCR6 and investigated the role of CXCR6 in mediating trafficking of leukocytes across hepatic endothelium.
Methods: Explanted liver tissue was obtained from the transplant program at University Hospitals Birmingham NHS Foundation Trust. Expression of CXCR6 and CXCL16 were analysed by rt-PCR, immunohistochemistry and flow cytometry. The role of CXCR6 in lymphocyte trafficking was analysed in a dynamic flow assay system with CO335224–4B, a small molecule inhibitor of CXCR6 (ChemoCentryx Inc, USA).
Results: Gene expression of CXCL16 was increased on bile ductules and endothelium in inflammatory liver disease, with the highest expression seen in in PBC. Serum CXCL16 concentration did not differ significantly between healthy controls and patients with chronic liver disease, except for patients with PBC who had significantly lower concentrations of circulating CXCL16 ($p < 0.001$). CXCR6 expression was enriched on liver-infiltrating lymphocytes (2-way ANOVA compared to blood $p < 0.001$). In cirrhosis, T lymphocytes and NKT cells showed significantly reduced CXCR6 expression compared to normal liver, whereas NK cell CXCR6 expression tended to increase. NK CXCR6 expression was associated with $CD56^{hi,}$ $Nkp30^{+}$ and $CD16^{-}$ expression, indicating an effector phenotype. NK CXCR6 expression was highest in PBC (one-way ANOVA $p = 0.03$). Inhibition of CXCR6 with a small molecule inhibitor reduced transendothelial migration of NK cells across hepatic endothelium under flow (Two-way ANOVA $p < 0.001$).
Conclusions: CXCR6 is enriched on effector NK cells in the inflamed human liver. This is particularly marked in PBC, where highest CXCL16 expression was also observed. Interestingly patients with PBC had lower peripheral CXCL16 concentration, which may be due to sequestration in the liver. A small molecule inhibitor of CXCR6 inhibited NK cell migration demonstrating the ability of CXCR6 to mediate NK cell recruitment though human hepatic endothelium. CXCR6 may be a therapeutic target in chronic inflammatory liver disease.

P0463
INFLUENCE OF KILLER CELL IMMUNOGLOBULIN-LIKE RECEPTORS (KIRS) AND THEIR HLA LIGANDS ON VERTICAL TRANSMISSION AND CHRONICITY OF HEPATITIS C VIRUS IN CHILDREN

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Background and Aims: There is adequate evidence on the vertical transmission (VT) of the maternal viral load of hepatitis C virus (HCV) during delivery and on HIV coinfection, but they do not explain all cases. The objective was to study the role of the immunogenetic profile (HLA, KIR, and KIR-ligand binding) of mothers and children in HCV-VT and chronicity
Methods: The study included 98 HCV-RNA(+) mothers and their 120 children. Among the 24 children infected, the disease became chronic in 8 and the virus was cleared in 16. We determined HLAclassI (A, B, Cw), HLAclassII (DRB1, DQA1, DQB1, DPA1, DPB1), Bonferroni-corrected P-value (Pc), and 16 KIRs (KIR2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, and 3DS1), by Luminex.
Results: *VT study:* Children with KIR2DL3 have a lower risk of infection (OR: 0.07, P = 0.019). The presence in the mother of Cw*06 (OR: 5.8, P = 0.007, Pc = 0.07) and Cw*0602 (OR: 5.7, P = 0.007, Pc = 0.08) increases the risk of infection of the child. Regarding C1 and C2 ligands, the presence of HLA-C1 in mothers and/or their children reduced the risk of infection (mothers: OR: 0.2, P = 0.009, children: OR: 0.3, P = 0.042), whereas the presence of HLA-C2 ligand increased the risk (mothers: OR: 6.2, P = 0.019, children: OR: 3.3, P = 0.042). Among the statistically significant associations found, we highlight that KIR binding to C1 ligand protected against VT and KIR binding to C2 ligand favored VT.
Chronicity: The presence in the mother of DQA1*01 (OR: 2.1, P = 0.009, Pc = 0.036), KIR2DS1 (OR: 7.2, P = 0.042) or KIR3DS1 (OR = 13, P = 0.013) favors chronicity in the child. The presence of allele DQB1*03 (OR: 0.05, P = 0.012, Pc = 0.048) and KIR2DS3 (OR: 0.5, P = 0.013) in the child and homozygosity for KIR3DL1/3DL1 (OR: 0.08, P = 0.0013) and for HLA-Bw4/Bw4 ligand (OR: 0.06, P = 0.022) were associated with viral clearance, whereas the binding of KIR3DS1 with Bw4 (OR = 7.2, P = 0.042), the binding of KIR2DS1 with C2 (OR = 7.2, P = 0.042), and heterozygosity for KIR3DL1/3DS1 (OR = 13, P = 0.013) favored viral chronicity.
Mother/child allele concordance: In the joint analysis of all HLAs, higher concordance was found between mothers and children with chronic infection vs those who had cleared the virus (67%±4.06 vs 57%±1.34, P = 0.045).

Conclusions: Studies of genetic factors in mothers and newborns are necessary to understand the processes underlying VT and the risk of chronic infection in the newborn. It can be affirmed that type C1 and C2 HLA ligands and their binding to KIRs are related to VT, while HLA-Bw4 ligands are associated with viral chronicity

P0464

KNOCKDOWN OF Gpbar1 (TGR5) RENDERS MICE HIGHLY SUSCEPTIBLE TOWARDS *Listeria monocytogenes* INFECTION

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Background and Aims: TGR5 (Gpbar1) is a membrane-bound bile acid receptor expressed in macrophages of several organs such as lung, liver and intestine as well as in peripheral blood mononuclear cells [1–3]. Stimulation of TGR5 inhibits the nuclear translocation of p65 and NF-κB transcriptional activation [3,4], thus reducing LPS-mediated production of pro-inflammatory cytokines [1,2]. Aim of the present study was to determine the role of TGR5 in an animal model for pathogen defense.
Methods: Male, 8–12 week old TGR5 knockout (KO) and WT mice were injected intravenously with 8×10^4 CFU/ml *L. monocytogenes* and observed for 7 days. On the 3rd day after infection with 2.5×10^4 CFU/ml bacterial titers were determined in spleen and liver. Serum levels of AST, ALT and LDH were measured using Spotchem-analyzer. Cytokine levels were determined in serum using Luminex cytometric bead assay. HE staining was carried out for all liver samples.
Results: Following L. monocytogenes infection TGR5 KO displayed a 50% mortality rate within a week of inoculation, while 90% of the TGR5 WT animals survived. In addition higher *L. monocytogenes* titers were detected in liver and spleen of TGR5 KO than in WT animals and HE staining revealed significantly more inflammatory infiltrates in livers from TGR5 KO mice. Accordingly, the levels of AST/ALT and LDH were elevated in serum from TGR5 KO animals as compared to the WT littermates. Furthermore, TGR5 KO mice had increased serum cytokine levels after *L. monocytogenes* infection as compared to WT mice.
Conclusions: Compared to WT littermates, TGR5 knockout mice showed higher *Listeria monocytogenes* titers in liver and spleen despite elevated serum inflammatory cytokines, such as TNFalpha. Accordingly, TGR5 KO mice were unable to control bacterial infection and systemic inflammation and thus displayed a significantly increased mortality rate.

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P0465

PERSISTENT INTRAHEPATIC Vγ9Vδ2 T-CELLS IMPAIRMENT IN HCV-INFECTED PERSONS

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Background and Aims: δγHepatitis C virus (HCV) persistence in the host results from inefficiency of both innate and adaptive immune responses to eradicate the infection. Among innate immune cells, a functional impairment of peripheral Vγ9Vδ2 T-cells was described during chronic HCV infection; this functional defect can be partially restored by using IFN-α, opening the possibility to boost antiviral innate immune response. Unfortunately, no data are available on intrahepatic Vγ9Vδ2 T-cells that instead, may represent the key actors in the anti-HCV response. Aim of our work was to compare intrahepatic and peripheral Vγ9Vδ2 T-cells in chronic HCV-infected patients (HCV) and in healthy donors (HD) subjected to gut resection surgery.
Methods: Phenotypic and functional features of intrahepatic and peripheral Vγ9Vδ2 T-cells were analyzed in 17 chronic HCV patients and in 14 HD by flow cytometry and ELISA assay.
Results: Irrespective of HCV infection, intrahepatic compartment was characterized by a lower frequency of Vγ9Vδ2 T-cells than in the peripheral blood. Although expressing an effector/activated phenotype, intrahepatic Vγ9Vδ2 T-cells showed a drastic reduction of their ability to produce IFN-γ after specific stimulation both in HCV and in HD subjects. Nevertheless, while intrahepatic Vγ9Vδ2 T-cells from HD can be functionally restored by using IFN-α co-stimulation, intrahepatic Vγ9Vδ2 T-cells from HCV-patients are refractory also to IFN-a co-stimulation, suggesting an exhaustion profile. Accordingly, Vγ9Vδ2 T-cells from HCV-patients presented a higher PD-1 expression than HD both in peripheral and intrahepatic compartments, probably due to persistent antigenic stimulation.
Conclusions: Overall, intrahepatic Vγ9Vδ2 T-cells from HCV patients presented an exhaustion phenotype and are functional impaired also after IFN-α co-stimulation. New studies are mandatory in order to identify molecular mechanisms inducing this functional anergy and to discover pathway able to restore Vγ9Vδ2 T-cell functionality.

P0466

IL-33 KNOCK OUT MICE ARE SENSITIZED TO SEVERE ConA LIVER INJURY BUT NOT CCl_4-MEDIATED LIVER INJURY

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Background and Aims: IL-33/ST2 axis play a protective role during acute hepatitis but little is known about the functional role of endogenous IL-33 in liver patho-physiology. We aimed to decipher the functional role of IL-33 by using IL-33 deficient mice during immune cell mediated and hepatotoxic driven liver injury.
Methods: We used a genetic model of acute hepatitis by using IL-33 deficient mice in ConA (a T cell-mediated hepatitis) and CCl_4 (a hepatotoxic agent-induced hepatitis) induced acute liver injury. The liver functions (AST/ALT), signature of cytokines and characterization of infiltrate cell in WT and IL-$33^{-/-}$ mice were carried out by biochemistry, qPCR and flow cytometry analyses.
Results: Our results demonstrated that IL-$33^{-/-}$ mice exhibited more severe ConA liver injury than WT mice evidencing a protective effect of IL-33 in this hepatic model while no difference was

observed in CCl_4-hepatitis between WT and IL-33$^{-/-}$ mice. The ConA-induced hepatic injury was associated with increased TNF-α, IL-1-β, IFN-γ and IL-6 cytokines in WT and IL-33$^{-/-}$ mice. The level of TNF-α and IL-1-β but not of IFN-γ and IL-6 was significantly higher in IL-33$^{-/-}$ mice than WT control. The intra-hepatic percentage of NK, NKT cells, T cells and B cells was not altered significantly between WT and IL-33$^{-/-}$ mice following ConA-hepatitis.
Conclusions: We evidenced that the genetic ablation of IL-33 sensitized the mice to severe ConA liver injury but not CCl_4-mediated liver injury.

P0467

PHENOTYPIC DISTORTION AND FUNCTIONAL ANOMALY OF LIVER DENDRITIC CELLS IN PRESENCE OF SUPPRESSOR MYELOID CELLS IN A MURINE MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Although non-alcoholic fatty liver disease (NAFLD) is characterized by increased inflammatory mucosal milieu, it is also associated with impaired antigen-specific immunity as evidenced by low response to hepatitis B surface antigen (HBsAg)-based vaccines. We checked the mechanisms underlying this in a murine model of NAFLD and exploring the functional capacities of most potent antigen-presenting cells, dendritic cells (DC).
Methods: A murine model of NAFLD was produced by feeding C57BL/6 mice with high-calorie and high-fat diet (520 calories/100 g, 50% fat). Control C57BL/6 mice received normal laboratory chow (360 calories/100 g, 13% fat). Hepatic DC, liver non-parenchymal cells (NPC), immunogenic myeloid cells and suppressor myeloid cells were isolated from murine liver by using density gradient and flow cytometry. Hepatic DC were cultured with liver NPC, immunogenic myeloid cells expressing $CD11b^+$ GR1− and $CD11b^+$ $GR1^+$ suppressor myeloid cells for 48 hours. Subsequently, DC were retrieved from the culture by magnetic-activated cell sorting. Subsequently, the expressions of costimulatory antigens on DC, production of cytokines by DC, and activation of HBsAg-specific lymphocytes by DC were evaluated.
Results: DC cultured with liver NPC and immunogenic myeloid cells had almost similar potential to activate HBsAg-specific T cells in antigen-specific manner and induced comparable levels of proinflammatory cytokines (IL-12, TNF-alpha, and IFN-gamma). However, DC cultured with suppressor myeloid cells of NAFLD mice had significantly lower capacity to present HBsAg to T cells and to induce proliferation of HBsAg-specific T cells ($p < 0.05$). Significantly lower levels of IL-12, TNF-alpha, and IFN-gamma were produced and significantly lower levels of CD86 and CD80 were expressed by DC cultured with liver-derived suppressor myeloid cells compared to DC co-cultured with liver NPC and liver-derived immunogenic myeloid cells ($p < 0.05$). In vivo, NAFLD mice produced significantly lower levels of antibody to HBsAg (anti-HBs) and induced lower magnitudes of HBsAg-specific T cells being immunized by HBsAg compared to those produced by control mice ($p < 0.05$).
Conclusions: Among different immunocytes, myeloid suppressor cells expressing $CD11b^+$ $GR1^+$ appears to down regulate DC function and compromise antigen-specific immunity and response to HBsAg-based vaccination in NAFLD.

P0468

NEGATIVE IMPACT OF HBV/HCV COINFECTION ON HBV OR HCV MONOINFECTION: DATA FROM THE FRENCH COHORT – ANRS CO22 HEPATHER

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Background and Aims: The ANRS CO22 HEPATHER ["Therapeutic options in a French cohort of HBV and/or HCV hepatitis"] is a multicentre cohort (32 centres) aiming to include 15,000 HCV- and 10,000 HBV-infected patients with a centralized biological collection and a specific information system. Given the limited number of non-Asian data on HBV/HCV coinfection, we compared the baseline characteristics of HBV/HCV coinfected patients in this cohort with those of HBV or HCV mono-infected patients.
Methods: 14,698 patients were included on November 19th 2014. Of 1099 patients with both HBV and HCV markers, 92 of the 93 patients with markers of active HBV and HCV infection (B/C patients) were analyzed and matched, each with 4 patients HBV- and 4 HCV-mono-infected patients on age, gender and duration of infection.
Results: Results are summarized in the Table. In B/C patients,
1. metropolitan origin (48%) was less prevalent than in C patients (63%) and more prevalent than in B patients (29%);

2. the prevalence of naive patients (49%) was higher than in C patients (33%);
3. overweight, obesity were less frequent and arterial hypertension more frequent than in B patients;
4. extensive fibrosis and cirrhosis (F3/F4 stages) (42%) were comparable to C patients (40%) and more frequent than in B patients (15%, p < 0.001);
5. a low rate of HBV DNA (<50 IU/mL) more frequent than in B patients (87% vs 62%, p < 0.001).

Table: Comparison of the characteristics of HBV/HCV coinfected and HBV or HCV matched mono-infected patients in the HEPATHER cohort at baseline

	BC (N = 92)	C (N = 368)	P
HCV treatment-naive	49%	33%	0.01
Metropolitan origin	48%	63%	0.01
Genotype 1 / 3	63% / 15%	63% / 14%	NS
F4 / F3 fibrosis stage	31% / 11%	33% / 7%	NS
Suspected cirrhosis	34%	42%	NS
Obesity/overweight	14%	21%	NS
Diabetes	15%	14%	NS
Arterial hypertension	34%	27%	NS
Undetectable viral load at enrolment Median 6.095 log	43%	49%	NS
Treated	29%	38%	NS
Metropolitan origin	48%	29%	<0.001
Delta-Coinfection	3%	3%	NS
F4 / F3 fibrosis stage	31% / 11%	9% / 6%	<0.001
Suspected cirrhosis	34%	13%	<0.001
Obesity/overweight	14%	26%	0.028
Diabetes	15%	10%	NS
Arterial hypertension	35%	24%	0.05
HBV DNA <50	87%	62%	<0.001

Conclusions: HBV co-infection was not associated with the severity of HCV-associated chronic hepatitis. In contrast, HCV co-infection harmfully impacted on fibrosis and reduced HBV replication in B/C co-infected patients. Thus, HCV treatment initiation must be prioritized in patients with an HBV/HCV coinfection, regardless of the severity of the liver disease.

P0469
MARKERS OF TISSUE REPAIR AND CELLULAR AGING ARE INCREASED IN LIVER TISSUE OF PATIENTS WITH DUAL CHRONIC HIV/HCV INFECTIONS

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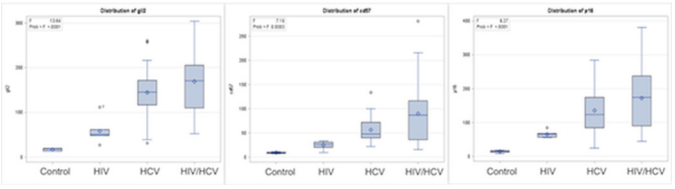
Background and Aims: When compared to HIV-uninfected persons, treated HIV-infected patients have an excess risk of chronic conditions, including liver disease, and experience excess liver related morbidity and mortality. Because fibrosis is an age-associated disease process and one regulated by activation of the fetal morphogen, Hedgehog (Hh) signaling, we compared histologic activity of markers for tissue repair, fibrogenesis and cellular aging in patients with HIV, HIV/HCV, and HCV infections.
Methods: Formalin-fixed liver tissue sections were identified for HIV/HCV, HIV, and HCV patients and non-infected controls. Patients were matched by age, gender, race/ethnicity, CD4 absolute (HIV infection), HIV viral suppression (HIV infection), and METAVIR stage of liver fibrosis (HCV infection). Specimens were stained with antibodies against (1) components of the Hh pathway [Sonic Hh (Shh) ligand, Glioblastoma 2 (Gli2), Patched (Ptc)] and ASMA to assess liver repair; (2) resident T lymphocytes (CD3, CD8, CD56, CD28), CXCL16 (NKT cytokine); (3) markers of cellular aging (CD57 and p16INK4a). Student's t test and ANOVA were used to compare histologic changes across the study groups.
Results: Overall 59 liver specimens were identified: 5 non-infected, 7 HIV, 25 HCV, 27 HIV/HCV patients. The cohort was 45% female, 54% black, and mean age at liver biopsy was 46 years (±8.7). Thirty-two percent of patients had severe liver fibrosis (METAVIR stage 3/4). HIV patients had a median CD4 of 651 cells/mm3 (±386) and 79% were on ARVs. All infected patients had greater expression of markers of tissue repair and fibrogenesis (Gli2 p < 0.0001, Shh p < 0.0001, Ptc p = 0.0214, ASMA p < 0.0001), T cell populations (CD3 p < 0.0001, CD8 p = 0.0362, CD28 p < 0.0001, CD56 p < 0.0001) and cytokines (CXCL16 p < 0.0001), and markers of cellular aging (CD57 p = 0.0003 and p16INK4a p < 0.0001) than non-infected controls (Figure). Incremental increases in histologic activity from HIV to HCV to HIV/HCV co-infection were identified for many markers (Figure).
Conclusions: Patients with HIV infection have increased markers of liver tissue repair and fibrogenesis, T cell populations including NK cells, and increased markers of cellular aging. This is true regardless of HCV co-infection. The additive effect of HIV and HCV co-infection was evident for a majority of the markers suggesting that patients with HIV/HCV have a greater fibrogenic response to tissue injury and more severe immune dysregulation which may result in more rapid cellular aging and age related disease.



P0470
DEVELOPMENT OF A NOVEL IGRA ASSAY TO TEST T CELL RESPONSIVENESS TO HBV ANTIGENS IN WHOLE BLOOD OF CHRONIC HEPATITIS B PATIENTS

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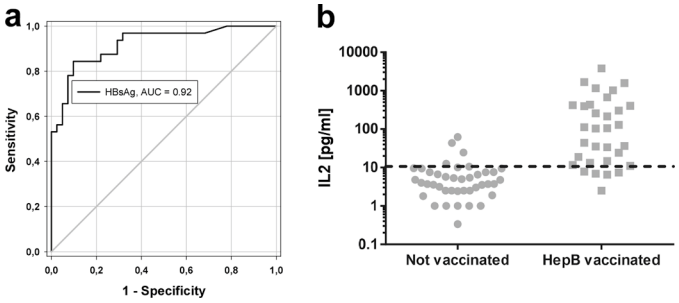
Background and Aims: Interferon gamma release assays (IGRA) have been developed to support the easy and fast diagnosis of diseases like tuberculosis, and CMV in transplant patients. IGRAs focus on cellular immunity especially memory T cells and thus also allow rapid screening prior to complex flow cytometric testing. We developed a novel, sensitive whole blood based cytokine release assay capable of assessing T cell responsiveness to HBV antigens in Hepatitis B patients and assessing hepatitis B vaccination status in healthy individuals.
Methods: 72 chronic Hepatitis B patients (CHB), 8 acute hepatitis B patients (AHB), 3 hepatitis B resolvers (RHB) and 80 healthy controls (HC) were tested by ELISA for IFNγ- and IL2-secretion in whole blood after challenge with synthetic peptide libraries of Hepatitis B core antigen (HBcAg) or Hepatitis B surface antigen (HBsAg). D-Glucose was supplemented to enhance cytokine secretion.
Results: The developed IGRA test reliably differentiated between Hepatitis B patients, vaccinees and unvaccinated healthy controls. CHB patients showed a weaker IFNγ response to HBcAg (52±16 pg/ml) compared to the AHB and RHB groups (97±39 pg/ml and 116±29 pg/ml, respectively), whereas HC remained unresponsive (13±2 pg/ml). IL2 levels after HBcAg challenge were also higher in the AHB and RHB groups compared to CHB and HC (50±21 and 312±143 pg/ml vs. 15±5 and 14±9 pg/ml, respectively). HBsAg stimulation led to increased IFNγ and IL2 levels in the AHB group (48±13 and 24±12 pg/ml) and even higher

levels in HC due to a high hepatitis B vaccination rate (49±10 and 170±59 pg/ml). CHB patients developed weaker IFNγ or IL2 responses to HBsAg (34±13 and 17±8 pg/ml, respectively). For HC, IL2 release after HBsAg stimulation depicted hepatitis B vaccination status with a diagnostic sensitivity and specificity of 85% and 90%.
Conclusions: Our novel IGRA whole blood based cytokine release assay constitutes an easy and robust tool for screening HBV specific cellular immunity in comparison to flow cytometry or ELISPOT.



P0471
NKp46 AND TRAIL ARE THE MAJOR NK CELL RECEPTORS INVOLVED IN ANTI-HCV ACTIVITY OF NK CELLS IN VITRO
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Background and Aims: Accumulating evidence indicates a pivotal role of natural killer (NK) cells for the early control and natural course of HCV infection. Various NK cell receptors have been implicated in anti-HCV activity but their individual contribution remained unclear.
Here, we show that NKp46 and TRAIL are the major NK cell receptors involved in anti-HCV activity of NK cells *in vitro*.
Methods: 14 healthy and 9 HCV-infected individuals were enrolled into this study. NK cells were isolated by negative selection using the MACS human NK cell enrichment kit (Miltenyi). Anti-HCV activity of IL-2 or IFN-a stimulated NK cells was studied in the presence or absence of blocking antibodies specific for CD54, 2B4, NKp30/44/46/80, NKG2D, TRAIL, and DNAM, respectively, using the HCV HUH7 replicon model.
Results: In IFN-a stimulated NK cells from healthy donors blocking of TRAIL (40%) had the strongest impact on anti-HCV activity, followed by NKp46 (35%), and NKG2D (11%). Of note, simultaneous blockade of TRAIL and NKp46 synergistically decreased anti-viral NK cell activity and displayed the strongest effect of all tested antibody combinations. In contrast, blockade of receptors on NK cells from HCV infected patients is less effective and hierarchy is altered: NKp46 (30%) > TRAIL (22%) > NKG2D (9%).
Analyzing IL-2 stimulated NK cells TRAIL (21%) > NKp46 (20%) > DNAM (8%) to have a significant effect on anti-HCV activity. Concurrent blocking of NKp46 and TRAIL had the strongest impact on NK cell function. Surprisingly, blocking these receptors on IL-2 stimulated NK cells of HCV infected donors is more effective: NKp46 (42%) > TRAIL (28%) > DNAM (17%).
Conclusions: Our data indicate that a variety of different NK cell receptors may play a role in anti-HCV activity and indicate an especially important role for NKp46 and TRAIL. In HCV infection activity of NKp46 is more effective than TRAIL, irrespective of IL2 or IFN-a stimulation.

P0472
ACCUMULATION OF PLATELETS IN THE LIVER MAY BE AN IMPORTANT CONTRIBUTORY FACTOR TO LIVER INJURY IN CHRONIC HEPATITIS B VIRUS INFECTION
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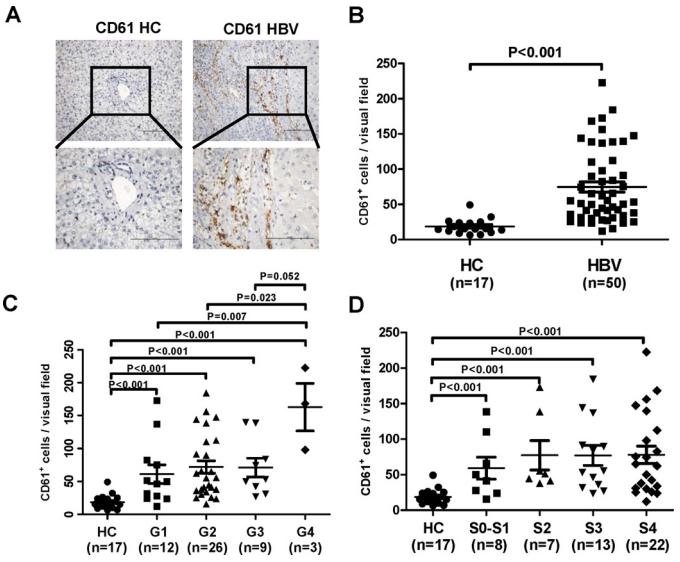
Background and Aims: Besides haemostatic properties, platelets have the features of inflammatory cells. Platelets have been shown to be contributed to the pathogenesis of acute liver damage of self-limited viral hepatitis and hepatitis B virus (HBV)-associated liver cancer in mouse models. However, the association between the platelets and chronic HBV infection remains largely unknown. We tried to clarify whether an accumulation of platelets in the liver contributes to liver injury and fibrosis in chronic HBV infection.
Methods: Fifty patients with chronic HBV infection who underwent liver biopsy were included. Seventeen healthy liver tissue samples were obtained from donors whose livers were used for transplantation. The platelets (marked by CD61) in the liver tissues were identified by immunohistochemistry. Nonspecific inflammatory cells such as CD68+ macrophages and monocytes in the liver of patients were also measured by immunohistochemical staining. According to the modified histology activity index described by Scheuer, the degree of hepatic inflammation and fibrosis of liver fibrosis in patients with chronic HBV infection was graded.



Results: Patients with chronic HBV infection had a significantly more extensive CD61+ platelets in the liver tissues compared to healthy controls (74.68±7.38 vs. 18.69±2.59 /HPF, $P < 0.001$). Patients with chronic HBV infection with higher inflammatory grading (G) scores had more CD61+ platelets in their livers compared to those with lower scores ($P < 0.05$). However, no association between liver platelets and fibrotic staging (S) scores was found in the patients ($P > 0.05$). The platelets in the liver tissues were strongly positively correlated with the nonspecific inflammatory cells such as CD68+ macrophages ($r = 0.625$, $P < 0.0001$) and MAC387+ monocytes ($r = 0.780$, $P < 0.0001$). The platelets in the liver tissues of patients with chronic HBV infection

were also positively correlated with alanine transaminase (r = 0.325, P = 0.023) and total bilirubin levels (r = 0.292, P = 0.042).
Conclusions: The accumulation of platelets in the liver may be involved in hepatic injury of patients with chronic HBV infection. Platelets may accumulate in the liver and take part in the pathogenesis of the liver injury in chronic HBV infection through the mechanism involving the nonspecific inflammatory cells such as macrophages and monocytes.

P0473
THE HEPATITIS C VIRUS MODIFIES CXC CHEMOKINE EXPRESSION INDUCED BY INFLAMMATORY CYTOKINES AND FACILITATES THEIR PRODUCTION IN RESPONSE TO EPIDERMAL GROWTH FACTOR

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Background and Aims: HCV has evolved powerful mechanisms to manipulate antiviral and innate immunity and to interfere with the inflammatory host response. Thereby the local composition of inflammatory and immune cells is largely determined by the pattern of chemokines released from the different cell types of the liver. The study investigates the influence of HCV on basal and inducible expression of the CXC chemokines CXCL1 to 3 and 8 in response to inflammatory cytokines and epidermal growth factor.
Methods: Huh-7 cells either infected with the HCV cc strain JC1 or harbouring the HCV subgenomic replicon were used to analyse the impact of HCV on cellular signalling and basal as well as inducible chemokine expression.
Results: HCV enhances TNFα- and IL-1β-inducible expression of CXCL1, 2 and 8. Surprisingly, HCV further facilitates EGF to induce the production of CXCL1 to 3 and 8 while EGF was almost incapable to do so in the absence of HCV. Thereby inhibitor studies and gene knockdown by siRNA suggest that the expression of CXCL-8 requires the activation of MEK1 and NF-κB but also depends on the sensitization of the EGF receptor (EGFR) tyrosine kinase activity, which is due to the HCV-dependent reduction of TC-PTP, an endogenous negative regulator of EGFR. Most interestingly, the data further suggest that HCV enhances basal expression of these chemokines via induction of an EGF-dependent autocrine circuit.
Conclusions: The data presented indicate, that HCV enhances TNFα- and IL-1β-inducible production of CXCL1, 2, 3 and 8 and extends the spectrum of EGF target genes towards these chemokines. The latter mechanism is employed by HCV to enhance basal chemokine expression via an EGF-dependent autocrine circuit.

P0474
CHARACTERISATION OF THE IMMUNE PROFILES OF CHRONIC HEPATITIS B PATIENTS FOLLOWING NUC DISCONTINUATION BY CyTOF MASS CYTOMETRY

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Background and Aims: It is currently not possible to predict whether the discontinuation of nucleos(t)ide analogues (NUCs) in Chronic Hepatitis B virus (HBV) patients will be followed by viral relapse and hepatic flares (HF). Through the utilisation of cytometry time of flight (CyTOF), a novel mass cytometry technology, in conjunction with traditional flow cytometry tools, we aim to establish an immune profile associated with the onset of HF upon therapy discontinuation.
Methods: Cytokine and chemokine serum levels (IL-1β, IL-6, TNF-α, IL-10, CXCL-8, CXCL-10) were measured longitudinally using Luminex in chronic HBV patients who developed HF (n = 6) and those who did not (n = 2) upon discontinuation of NUCs. HBV-specific T cell responses were assessed after *in vitro* expansion of patient peripheral blood mononuclear cells with overlapping HBV peptides pools. An in-depth longitudinal analysis of the expression of 35 markers involved in the activation, differentiation, exhaustion and anti-viral effector function of T and NK cells is performed by CyTOF mass cytometry.
Results: The rise in serum ALT following viral relapse after therapy discontinuation are associated with a significant increase in the serum levels of CXCL-10 and IL-10. Despite comprehensive analysis of HBV-specific T cells using HBV peptides, functional antigen-specific T cells in the periphery were not detected. Preliminary analysis by CyTOF reveals significant differences in the frequency and functionality of T and NK cell populations longitudinally (during and after discontinuation of therapy), between patients characterised by viral relapse and those with immune control.
Conclusions: Consistent with previous studies, HF are associated with increased serum levels of CXCL-10 and IL-10 while HBV-specific T cells remain undetectable in the periphery. The absence of circulating virus-specific CD8+T cells during HF suggests that hepatic inflammation following NUC withdrawal in chronic HBV patients is induced independent of virus-specific CD8+ T cells. CyTOF technology allows us to investigate an unprecedented number of parameters (currently up to 42) for a more detailed assessment of biomarkers associated with sustained immune control of HBV.

P0475
POST-LIVER TRANSPLANT FIBROSIS ALLOGRAFT PROGRESSION IS ASSOCIATED WITH INCREASED LEVELS OF SOLUBLE NKG2D LIGANDS

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Background and Aims: Histological abnormalities are commonly present in late post-transplant biopsies, including variable stages of fibrosis. Fibrosis proved to be strongly related to transplant-related factors in several studies. The aim of our study was to investigate clinical and immunological risk factors for different stages of fibrosis occurrence following LT.
Methods: A total of 174 LT recipients were enrolled in the study. Cytokines were assessed by flow cytometry with the "BD Cytometric Bead Array (CBA) Human Th1/Th2/Th17 Cytokine Kit". Assessment of soluble major-histocompatibility complex class I-related chain A (sMICA), soluble major-histocompatibility complex class I-related chain B (sMICB) and soluble UL16 binding protein 2 (sULBP2) was realized by enzyme linked immunosorbent assay. Screening for anti-HLA class I, class II, or MICA antibodies was performed using Luminex technology. Chi-square test was used for comparing categorical data and Kruskal–Wallis test was used for comparing continuous variables. Multiple regression analysis was performed to identify independent risk factors for progression of fibrosis after LT.
Results: The following variables were statistically significant different according to different stages of fibrosis (F0 to F4 METAVIR): higher donor age (p = 0.03), high serum levels of TNF alpha (p = 0.04), IL6 (p < 0.0001), presence of anti-HLA class I Ab

($p = 0.03$), higher serum levels of sMICA ($p = 0.04$), sMICB ($p = 0.007$) and ULPB2 ($p = 0.004$), presence of HCV ($p < 0.0001$), presence of recurrent cholangitis ($p = 0.02$). Presence of anastomosis stenosis ($p = 0.052$) and IL10 serum levels ($p = 0.058$) reached only marginal statistical significance. Time from LT to fibrosis stage evaluation did not differ between different stages of fibrosis. Mean time from LT to fibrosis evaluation was 66.9 months. Independent variables associated with progression of fibrosis following LT were: HCV infection, recurrent episodes of cholangitis, and higher serum levels of IL6, sMICA and presence of non-DSA HLA class I antibodies.
Conclusions: sMICA, IL6 serum levels, non-DSA HLA class I antibodies and recurrent episodes of cholangitis should prompt screening for fibrosis progression and may be useful for performing protocol liver biopsies also in non-HCV recipients.

P0476
EXPERIMENTAL INTERVENTION AGAINST CCL5/RANTES ATTENUATES LIVER FIBROGENESIS AND THE PROGRESSION OF HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma (HCC), the malignant transformation of hepatocytes, often arises on the basis of chronic liver inflammation, resulting in liver fibrosis. In this process, immune cells play a crucial role. In the current study, we investigated whether therapeutic intervention with Evasin-4 – a CCL5/RANTES inhibitor – affects the development of hepatic fibrogenesis. Additionally, we evaluated the role of CCL5/RANTES in liver fibrosis as well as HCC initiation and progression, in an experimental model of chronic liver injury.
Methods: Evasin-4 was injected i.p. daily in 6-week-old $NEMO^{\Delta hepa}$ mice for 2 months. Additionally, the role of CCL5/RANTES in the development of fibrosis and tumour initiation and progression was analysed in an experimental model of chronic liver injury by generating $NEMO^{\Delta hepa}/CCL5^{-/-}$ mice using hepatocyte-specific NEMO knockout ($NEMO^{\Delta hepa}$) and constitutive CCL5/RANTES ($CCL5^{-/-}$) mice. The progression of liver fibrosis and HCC development was evaluated with molecular biological methods (Western Blot, qRT-PCR and immunodetection).
Results: Pharmacologic intervention of the CCL5/RANTES pathway using Evasin-4 in $NEMO^{\Delta hepa}$ mice led to a significant improvement of liver fibrosis, as evaluated by Collagen IA1 and Sirius red staining. Furthermore fibrosis reduction was confirmed by markers of hepatic fibrosis and collagen deposition (αSMA, Collagen IA1, TGF-β, MMP-2 and MMP-3). One year-old $NEMO^{\Delta hepa}/CCL5^{-/-}$ mice exhibited improvement of hepatic fibrogenesis as assessed by protein analysis and immunodetection methods. No differences were found between $NEMO^{\Delta hepa}$ and $NEMO^{\Delta hepa}/CCL5^{-/-}$ mice regarding tumour initiation in the liver. However, $NEMO^{\Delta hepa}/CCL5^{-/-}$ mice displayed smaller and less malignant tumours compared with $NEMO^{\Delta hepa}$ mice. In addition, $NEMO^{\Delta hepa}/CCL5^{-/-}$ tumours displayed significantly reduced proliferation and less pronounced angiogenesis compared with $NEMO^{\Delta hepa}$ livers.
Conclusions: Therapeutic modulation of the CCL5/RANTES pathway with Evasin-4 significantly attenuated progression of of hepatic fibrosis. Deletion of CCL5/RANTES in $NEMO^{\Delta hepa}$ mice leads to the amelioration of hepatic fibrogenesis and HCC progression. These results indicate that CCL5/RANTES is an attractive target for the treatment of chronic liver disease.

P0477
FLUORESCENCE MICROSCOPY IDENTIFIES COMPLEX HETEROGENEITY OF ANTIGEN PRESENTING CELLS IN NORMAL HUMAN LIVER

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Background and Aims: Antigen presenting cells (APC) of the mononuclear phagocyte system (MPS) are an important population of cells involved in the tissue homeostasis and the immune response in autoimmunity, infection and cancer. APC have been thoroughly characterized in tissues such as the human skin and lymph node but despite growing evidence in animal tissue there is a dearth of evidence characterising APC in human liver [1]. Having an improved understanding of the composition of liver APC and their location within the liver lobule is vital to appreciating changes that occur in disease and identifying potential therapies to target them.
Methods: Normal liver tissue was obtained following resection of benign vascular malformations or from living donor liver transplant biopsies. Multicolour immunofluorescence microscopy (MIM) was performed on fresh frozen and formalin fixed paraffin embedded samples. Parenchymal, endothelial, epithelial and stellate cells were identified using a mixture of markers and APC were initially characterized using subset specific markers, then further characterized using a variety of chemokine receptors, Fc receptors, and scavenger receptors. All findings were observed on a minimum of three donors.
Results: MIM allowed for the accurate distinction between APC and non-APC cells. KCs were scattered throughout the liver lobule and uniformally expressed CD68, CD16, CD163, CD169, HAM-56, and CD206. KC were composed of (i) distinctly large spindle cell shaped cells located predominantly in zones 1, 2 and were $CCR2^{+}PU.1^{+}CD14^{+}CD64^{hi}CD45^{hi}$ and (ii) smaller circular $CCR2^{-}PU.1^{-}$ cells with a lower expression of CD45. Myeloid DC were located in the portal tracts and the liver hosted CD1c, CD141 and pDC subsets. Monocytes were found throughout the sinus were composed of a mixture of $CD14^{+}$ and $CD16^{+}$ cells, they also expressed CD36 and Lyve-1.
Conclusions: We have used MIM as a powerful tool to characterize the complexity of the liver lobule and identify hitherto unappreciate heterogeneity in human KCs that follows an acinar distribution. We have also identified the major DC and monocyte subets and as a result allowed for a means to further characterize these populations to identify changes that occur in disease and facilitate the identification of therapeutic targets specific to APC in the liver.

Reference(s)

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P0478

NKG2D-DEPENDENT CROSS-TALK BETWEEN NK CELLS AND CD4 T CELLS IN CHRONIC HEPATITIS B

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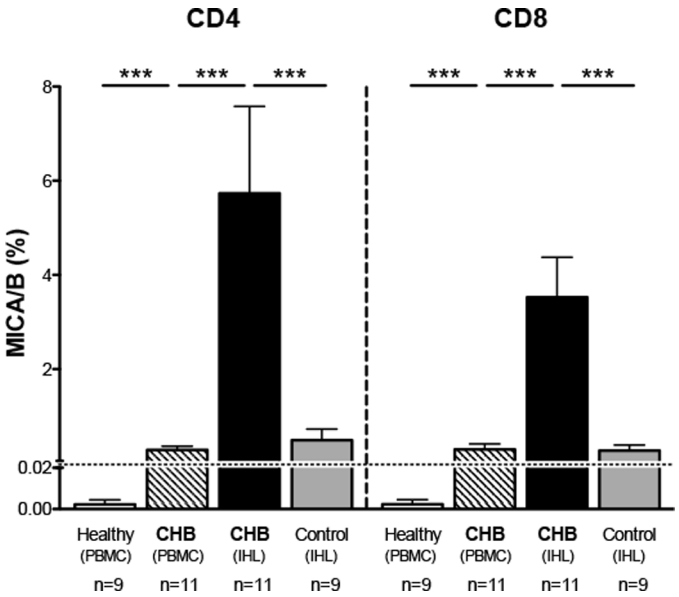
Background and Aims: NK cells are emerging as potent regulators of adaptive immunity; we have recently shown that they can kill hepatitis B virus (HBV)-specific T cells in a contact-dependent manner (Peppa JEM 2013). The TRAIL pathway was involved in NK cell deletion of T cells but blocking studies implicated additional pathways. In this study we explored the role of NKG2D-dependent interactions between NK cells and T cells.

Methods: We evaluated peripheral blood from healthy individuals and patients with chronic hepatitis B (CHB). Intrahepatic lymphocytes were isolated from liver tissue of biopsies (CHB) or resections (controls). A MICA-transfected B lymphoblast cell line, C1R MICA, was used to assess NK cell activation and cytotoxicity. Global, activated and HBV-specific T cells from the circulation and liver were stained with NKG2D-L mAb. NK cell depletion and NKG2D-blocking mAb were used for functional experiments.



Results: The activatory receptor NKG2D was maintained at high levels on NK cells from patients with HBV and further increased on intrahepatic NK cells. NKG2D-ligands (NKG2D-L) are not usually expressed on T cells but were upregulated on T cells (CD4>CD8) in CHB patients, particularly those with liver inflammation. NKG2D-L were preferentially expressed on activated and HBV-specific T cells, and strikingly enriched on T cells in the CHB liver compared to their circulating counterparts or to T cells in control livers without inflammation. Following NKG2D pathway blockade in vitro, there was a small but consistent rescue of circulating and intrahepatic HBV-specific CD4 T cells from CHB patients with ongoing liver inflammation. (NKG2D-L)-expressing cells were able to trigger activation and cytotoxicity of NK cells from patients with CHB in an NKG2D-dependant manner. We therefore concluded that the NKG2D pathway allows bidirectional cross-talk between T and NK cells. In support of this, the NKG2D+ fraction of NK cells were more activated (HLA-DR+) in patients with CHB than controls, particularly in the infected liver.

Conclusions: These results imply that NKG2D-L expressing T cells drive activation and cytotoxicity of NK cells in the HBV-infected liver, promoting deletion of HBV-specific T cells. Thus NKG2D-dependent CD4 T cell/NK cell interactions may support innate immunity at the expense of the adaptive arm in CHB.

P0479

NAIVE-LIKE CD8+ T CELLS SPECIFIC FOR SUBDOMINANT HEPATITIS B VIRUS EPITOPES ARE PRESENT IN CHRONICALLY INFECTED PATIENTS

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Background and Aims: Virus-specific CD8+ T cells are rarely detectable ex vivo by conventional tetramer stainings in chronically HBV-infected patients. This lack of HBV-specific CD8+ T-cell responses may be explained by insufficient priming or terminal deletion due to T-cell exhaustion.

Methods: To characterize HBV-specific CD8+ T cells not detectable by conventional methods we used a combination of tetramer-associated magnetic bead enrichment and multiparametric flow cytometry and performed phenotypic, functional and viral sequence analyses.

Results: By using this enrichment strategy we were able to detect 74 CD8+ T-cell populations specific for 4 HLA-A0201 restricted HBV epitopes (Core18–27, Env183–191, Env335–343 and Pol455–463) in 31 chronically HBV-infected patients suggesting that these cells are not completely deleted. Interestingly, a proportion of the analyzed CD8+ T cells specific for Env183–191, Env335–343 and Pol455–463 epitopes had a naïve-like phenotype (25%, 48% and 19% respectively) defined by high expression of CD45RA, CCR7 and CD27 and low expression of CD11a. By contrast, enriched CD8+ T cells specific for the immunodominant Core18–27 epitope predominantly showed an effector-memory phenotype (CD45RA-, CCR7-, CD27+, CD11a+). However, the presence of wild type sequences in patients with naïve-like HBV-specific CD8+ T cells indicated that the presence of naïve-like cells is not due to infection with a variant HBV containing mismatched epitopes. Moreover, the presence of HBV-specific CD8+ T cells with naïve-like phenotype did not correlate with any clinical parameter such as HBeAg status, viral load or transaminases and was independent of antiviral treatment. Further investigations will address the underlying mechanisms for possible priming defects and clarify whether intrinsic CD8+ T-cell defects, dysfunction of antigen-presenting cells, lack of CD4+ T-cell help or immunoinhibitory cytokines such as IL-10 may play a role in this setting.

Conclusions: Our results indicate that HBV-specific CD8+ T cells are not completely deleted in chronically infected patients, but are rather maintained at a very low frequency. Furthermore, a substantial fraction of CD8+ T cells specific for subdominant HBV epitopes display a naïve-like phenotype despite ongoing viral replication. This suggests that insufficient priming of HBV-specific CD8+ T cells may also contribute to CD8+ T-cell failure in chronic HBV infection and that therapeutic vaccination may be a feasible approach in this patient cohort.

P0480

ROLE OF THE IMMUNE MICROENVIRONMENT DURING HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatocellular carcinoma (HCC) is the 3rd cause of cancer-related death worldwide. Collective data evidenced the tumor microenvironment (TME) as a key actor during carcinogenesis. Using relevant liver tumor mouse models mimicking human beta-catenin-dependent tumorigenesis, we identified 2 critical interconnected effectors of the TME controlling this oncogenic process: the invariant Natural Killer T cells (iNKT) and LECT2. Their specific deletion leads to the emergence of highly invasive HCC associated with lung metastasis (Anson M et al., 2012).
Our aim is to address functionally the regulatory role of LECT2 on the inflammatory response driven by tumor development.
Methods: Preneoplastic ($Apc^{-/-}$) and tumoral (Lpk-myc) models (WT and LECT2 deficient) that recapitulate human beta-catenin-dependent HCC are used. Immune liver content is analysed by flow cytometry, cell sorting and immunohistochemistry. The expression of LECT2 is analysed from a large cohort of human HCC samples by TaqMan qPCR.
Results: We found a significant accumulation of myeloid cells ($CD11c^{-}$ $CD11b^{+}$ $F4/80^{+}$ $Ly6C^{hi}$ $Ly6G^{-}$) in HCC livers and impressively more in the absence of LECT2. These cells were found to express LECT2 receptor CD209a. We investigate the mechanism by which LECT2 exerts its effects using Bone-Marrow-Derived-Macrophages (BMDM) from WT and LECT2-deficient mice, pretreated with r-LECT2 and/or LPS. We found that (1) LECT2 regulates drastically LPS-induced TNFa secretion, (2) BMDM from LECT2-deficient mice are hypersensitive to LPS exposure compared to WT. Translated *in vivo* (HCC), myeloid cells exhibit immature phenotype evidenced by low level of MHC class II and CD1d that associated with a substantial increase in their ability to produce IL-10. These results suggest that LECT2 regulates the inflammatory response during HCC through a major targeting of myeloid cell functions that might impact subsequently on iNKT cells.
We evidenced that undifferentiated structures arising within LECT2-deficient tumor nodules harbor a molecular signature associated with EMT/metastasis. Finally, we found that LECT2 expression inversely correlates with tumor grades and the presence of significant inflammatory human HCC emphasizing its role in both differentiation and inflammation under oncogenic process.
Conclusions: Our data suggest that LECT2 controls functionally liver myeloid cells to regulate an inflammatory response compatible with the maintenance of hepatocyte differentiation.

P0481

GENOME WIDE MIRNA: MRNA INTEGROME ANALYSIS REVEALS KEY PATHWAYS, BIOLOGICAL PROCESSES AND GENE FAMILIES IN CD4+ T CELLS THAT DIFFERENTIATE VARIOUS STAGES OF HBV INFECTION

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Background and Aims: CD4 + T cell immune responses play an important role in pathogenesis during various stages of hepatitis B virus infection. However, the molecular mechanisms by which CD+ T cells exert their modulatory activity are largely unknown. We investigated the role of miRNA mediated regulatory control of mRNA expression and key pathways and molecular mechanism of CD4+ T cells at different stages of HBV infected patients.
Methods: Three groups of patients [Acute Viral Hepatitis, AVH-B (n = 4, mean age 45yr, M/F = 2); Chronic HBV with raised ALT (n = 9, HBeAg+ = 5, mean age 38yr, M = 6) and Chronic HBV with persistently normal ALT (n = 12, HBeAg+ = 3, mean age 34 yr, M = 7)] and matched controls (n = 11, mean age 33 yr, M = 6) were studied. Microarray based whole genome gene expression analysis and whole miRNA expression analysis of CD4+ T cells was performed using Agilent Platform. Differentially expressed transcripts (Genes and miRNAs) between patient groups and key pathways and biological processes regulated were identified by various statistical methods. Differentially expressed gene sets and their targeting differentially expressed miRNA along with pathways and biological categories regulated by them were subjected to Integrome analysis to identify disease baseline and stage specific signatures of HBV infection.
Results: We identified a total of 116 genes and 53 differentially expressed miRNAs in one or more of the infection stages. Integrome analysis revealed 28 key pathways, biological categories and gene families that were enriched in baseline infection and unique to specific infection stages harbouring the identified genes and miRNAs (Figure 1). Network modelling analysis revealed HBV infection stage specific and baseline clustering of pathways, gene families and key biological categories. Some of the biological events and gene families enriched include Apoptosis, Calcium Signalling, Key Cellular processes (Adhesion, Cell cycle, Differentiation, Migration, Proliferation), Cell surface, Chemokines, and Cytokines, Immune response, MAPK signaling, NFKB signalling and TGFbeta signalling pathway.
Conclusions: Integrome analysis revealed induction of unique cluster of miRNAs and repression of their target genes that differentiates acute and chronic infection stage, chronic infection with and without hepatic injury in CD4+ T cells.

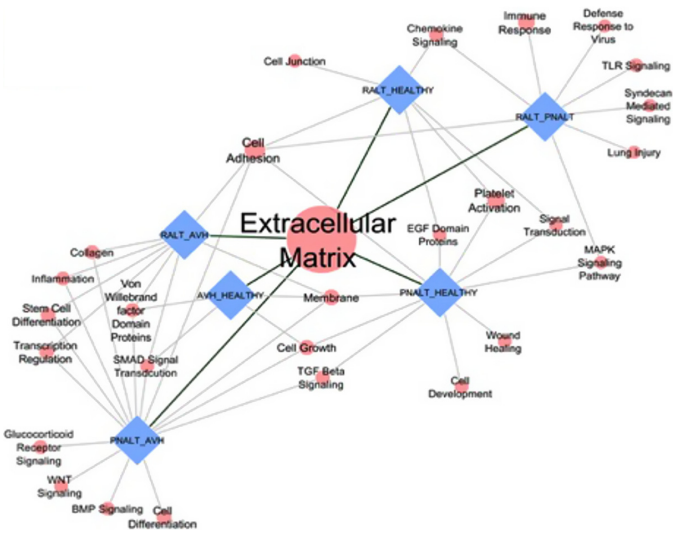

Figure 1. Key pathways and biological processes regulated by miRNA:mRNA in CD4+ T cells during different stages of HBV infection.

P0482

IMPROVING NATURAL KILLER CELLS CYTOTOXICTY AND HARNESSING HEPATOCYTE CANCER PROGRESSION BY MIR-486-5P

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Background and Aims: Insulin-like Growth Factor-1 Receptor (IGF1R) activation mainly through IGF-2 is a hallmark in Hepatocellular carcinoma (HCC), stimulating several mitogenic signaling pathways most importantly PI3K/Akt/mTOR pathway. Among the immune cells, IGF-1R showed high expression on Natural Killer (NK) cells, and it was also reported to have a prominent role in NK cell differentiation and proliferation mainly through IGF-1. NK cells are known to be the first native defender against HCC activated by NKG2D receptor, mediating its cytotoxicity mainly through perforins release. In our previous work, let-7a and miR-615-5p were proved to be tumor suppressors through targeting IGF-2. IGF-1R expression was found to be regulated by several miRNAs; however, miR-486-5p has never been investigated in HCC. So the aim of this study was to control the HCC tumor progression through the direct impact of miRNAs on NK cells as well as their target hepatocytes in an immunotherapeutic approach.
Methods: In-silico analysis was preformed to predict potential miRNAs targeting IGF1R. Huh7 cells were cultured and NK cells were isolated from 27 HCC patients. Both cell types were transfected by the miRNAs of interest using lipofection. Total RNA was extracted and quantified using qRT-PCR. Viability and proliferation analysis were performed using MTT and BrdU assays. Significance of results was determined by unpaired student t-test ($p < 0.05$) and graphed using Graph Pad Prism 5.0 software.
Results: Bioinformatic analysis predicted miR-486-5p, miR-615-5p and let-7a to target IGF-1R with high scores. Gain and loss of function of miR-486-5p, miR-615-5p and let-7a showed significant down regulation of both IGF1R and its downstream oncoprotein mTOR in Huh7 cells. Cellular viability and proliferation of Huh7 cells were repressed by miR-486-5p comparable to the validated tumor suppressors miR-615-5p and let-7a. Upon ectopic expression of miR-486-5p in NK cells of HCC patients both NKG2D and perforins expression were significantly elevated. However, a significant down regulation of IGF-1R was observed. Interestingly, IGF-1 which is a validated target of miR-486-5p was found to be significantly upregulated in NK cells of HCC patients.
Conclusions: MiR-486-5p is a novel tumor suppressor microRNA. In the current study, it was shown to have a dual role in enhancing NK cell cytotoxicity through inducing IGF-1 expression and harnessing the tumor progression of its target hepatocyte via IGF-axis repression.

P0483

CHANGES IN LIVER IMMUNITY UPON HBV INFECTION: A COMPARISON OF HUMANISED MICE AND HUMANS

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Background and Aims: The lack of robust animal models to study chronic HBV infection and immunopathogenesis has been a roadblock to the development of effective therapies. Humanised mouse models containing both human hepatocytes and immune system have been proposed as potential models for studying HBV pathogenesis. However, there has been little direct comparison of humanised mouse liver immunity with that of humans. Our aim is to compare how similar intrahepatic immune cells in HBV-infected humanised mice are to those from pathologic human livers.
Methods: Humanised mice containing both human hepatocytes and a matching immune system (HIL mice) were generated by reconstituting NOD-*scid IL2rg*$^{-/-}$ mice with CD34^{+} cells from human fetal livers. HIL mice were infected with either mock control or HBV, and their liver immune cells isolated at 4, 10 and 20 weeks post-infection. Intrahepatic immune cells were also isolated from perfused healthy and HBV-infected pathologic human livers. Intrahepatic immune cells from humanised mice and humans were analysed for immune cell frequency and functional responses.
Results: Despite high levels of intrahepatic immune reconstitution in humanised mice, immune subset frequencies of uninfected and HBV-infected HIL livers differed from healthy and HBV-infected human livers. In particular, HIL mouse livers had higher CD4/CD8 T cell ratios and lower frequencies of NK, γδ T and MAIT cells as compared to human livers. Furthermore, HBV-infected HIL mice did not show any expansion of HBV-specific T cells, that were not detected despite the use of a comprehensive pool of HBV peptides covering the whole HBV proteome in ELISPOT assays. However, the majority of HBV-infected but not mock-infected mice developed liver damage and robust human serum cytokines responses starting 8 weeks post-infection. The liver inflammatory events were linked with a specific enrichment of CD14+CD16+ inflammatory monocytes and dendritic cells that closely mimics what is seen in pathological human livers.
Conclusions: Although intrahepatic lymphocyte populations and virus-specific immunity could not be fully recapitulated in HBV-infected HIL mice, the intrahepatic inflammatory events triggered by HBV infection are similar to those detected in HBV chronically infected humans. We posit that this model can be exploited to disentangle the immunological mechanisms of chronic liver inflammation secondary to chronic HBV infection.

P0484

CD3(+)CD56(+) NK-LIKE T CELLS SHOW REDUCED ANTI-VIRAL ACTIVITY IN ACUTELY HCV/HIV INFECTED PATIENTS

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Background and Aims: In Western Europe and the US approximately 30% of HIV(+) patients are co-infected with the hepatitis C virus (HCV), which has become a major cause of morbidity and mortality. In HIV(−) patients CD3(+)CD56(+) NK-like T cells, a subset of innate lymphocytes, have been suggested to modulate outcome of acute hepatitis C. However, it remained unclear whether these cells display direct anti-HCV activity and how HIV co-infection may modulate functions of CD3(+)CD56(+) NK-like T cells.
Methods: 36 HIV(+) patients with acute hepatitis C (spontaneous clearance: n = 13; chronic course: n = 23) were studied. 8 HCV(+)/HIV(−) patients, 12 HIV(+) patients with chronic hepatitis C, 8 HIV(+)/HCV(−) patients and 12 healthy individuals were enrolled as controls. Frequency, phenotype and IFN-γ secretion was studied by flowcytometry. CD3(+)CD56(+) NK-like T cell-mediated inhibition of HCV replication was analyzed using the HuH7A2HCVreplicon model.

Results: Frequencies of CD3(+)CD56(+) NK-like T cells did not differ significantly between the study groups. Phenotypic analysis revealed that acutely HCV infected patients displayed a significantly lower frequency of CD161(+)CD3(+)CD56(+) NK-like T cells compared to controls. Of note, frequency of CD161-expressing cells was positively correlated with IFN-γ production. We found, IL-12/IL-15 stimulated CD3(+)CD56(+) NK-like T cells from healthy donors effectively block HCV replication in vitro. Supernatants of IL-12/IL-15 stimulated CD3(+)CD56(+) NK-like T cells also significantly inhibited HCV replication in vitro, suggesting that non-cytolytic mechanisms may play a major role. In addition, we only observed minimal killing of HuH7A2HCVreplicon cells by CD3(+)CD56(+) NK-like T cells and could confirm that blocking of IFN-γ with a specific antibody significantly reduced the anti-viral activity of these cells. However, when CD3(+)CD56(+) NK-like T cells from HIV(+) patients were studied we found HIV infection to be associated with a significantly impaired IFN-γ production, irrespective of HCV co-infection. In line with this observation, CD3(+)CD56(+) NK-like T cells from HIV(+) patients were significantly less effective in blocking HCV replication in vitro than cells from healthy individuals.
Conclusions: Taken together, our data indicate that CD3(+)CD56(+) NK-like T cells have the potential to block HCV replication but are functionally impaired in HIV(+) patients. This might represent a novel mechanism of dysregulated immune response in HIV/HCV co-infected patients.

P0485
FUNCTIONAL IMMUNE RESTORATION CORRELATES WITH HBsAg DECLINE AND MAY PREDICT TREATMENT RESPONSE ON SEQUENTIAL NUC THERAPY IN CHRONIC HEPATITIS B

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Background and Aims: Sequential/combined therapeutic approaches comprising Pegylated-Interferon (Peg-IFNα) and nucleot(s)ide analogues (NUC) are being given greater consideration as treatment strategies for chronic hepatitis B (CHB) to achieve HBsAg loss. We demonstrated boosting of NK cells in eAg^- patients treated with Peg-IFNα *(Micco et al, J. Hepatol, 2013),* and postulated this effect could be maintained with sequential NUC therapy. Differential NK cell responses in patients receiving sequential NUCs were analysed and correlated with HBsAg response, to elucidate a possible treatment advantage with Peg-IFNα exposure.
Methods: PBMC from 18 eAg^+ patients during Peg-IFNα therapy were utlised. 9/18 patients considered Peg-IFNα non-responders after 48-weeks therapy progressed to sequential NUCs and were followed until virally suppressed. Phenotypic and functional analysis of NK cell subsets was performed by multicolour flow-cytometry and findings correlated with on-therapy HBsAg changes.
Results: Peg-IFNα expanded $CD56^{bright}$ NK cells by 3-fold ($p = 0.0001$) which was maintained on sequential therapy. NK cell receptor expression was analysed. All receptors, except NKG2C, were maintained on sequential NUCs, with marked augmentation in the expression of NKp30 and NKp46 on $CD56^{bright}$ NK cells ($p \leq 0.05$). These NK cells maintained their ability to degranulate and produce IFNγ during sequential therapy, functional restorations not seen on NUCs alone ($p = 0.0001$ and 0.002 respectively). TRAIL expression was analysed; this decreased on sequential NUCs, but remained higher than baseline. 6/9 patients had significant declines in HBsAg (>0.5 $\log_{10}$ IU/ml) on sequential NUCs. We noted that only these patients showed the ability to increase the proportion of functional NK cells ($IFN\gamma^+$ and $CD107^+$) on sequential NUCs. Additionally only these responders demonstrated a reduction in TRAIL expression on sequential NUCs, compared to those without HBsAg decline, who showed the reverse.
Conclusions: Restoration of NK cell cytotoxic/effector functions is seen on sequential therapy, but only in those patients with HBsAg decline. Lower expression of TRAIL also correlates with treatment response, in line with our finding that $TRAIL^+$ NK cells can delete antiviral T-cells (*Peppa et al, JEM 2013*). IFNγ, CD107 and TRAIL expression on NK cells may predict those patients who are likely to demonstrate HBsAg decline on sequential therapy. Given these findings, the TRAIL pathway may be a potential future target in order to improve treatment outcomes in CHB.

P0486
HEPATIC APOPTOSIS INFLUENCED BY DEFICIENCY OF GROUP VIA CALCIUM-INDEPENDENT PHOSPHOLIPASE A2 LEADS TO PRO-INFLAMMATORY RESPONSE BY KUPFFER CELLS

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Background and Aims: Emerging data indicate that group VIA calcium-independent phospholipase A2 (iPLA2β) regulates macrophage chemotaxis towards apoptotic cells. We have previously shown that iPLA2β deficiency causes an accumulation of hepatocyte apoptosis which leads to increased inflammation. The aim of the present study was to determine whether hepatic apoptosis with iPLA2β deficiency would alter M1/M2 response of resident Kupffer cells (KC) and Th1/Th2/Th17 response in lymphocytes, and whether this response could be modified by CD95/FasL-induced liver injury in vivo.
Methods: KC and splenocytes were isolated from control (WT) and $iPLA2\beta^{-/-}$ male mice and were respectively stimulated by 1 μg/mL LPS or 10 μg/mL ConA in vitro. In another set of experiments, mice were injected with 0.125 μg/g body weight CD95/FasL antibody for 6 h. Cytokine expression and release were measured by qRT-PCR and ELISA. Histology and immunohistochemistry was performed on secondary lymphoid organs.
Results: KC isolated from $iPLA2\beta^{-/-}$ mice secreted lower levels of IL-6, TNF-α, IL-10, and in vitro LPS-stimulated KC from mutant livers released cytokines at strongly suppressed levels compared with WT KC (for example IL-6 release in mutant vs. WT: 4-fold vs. 12-fold increase). On the contrary, upon CD95/FasL-induced apoptosis in vivo, KC from the mutant livers released increased IL-6 while those from WT livers displayed suppressed secretion. LPS stimulation in vitro in mutant KC led to an increased release of IL-6 and TNF-α (mutant vs. WT: 5 and 16 vs. 3 and 5 fold increase for IL-6 and TNF-α, respectively). The release of IL-4 and TGFβ1 by KC was not markedly altered among WT and mutant livers. In secondary lymphoid organs, apoptosis in spleen and mesenteric lymph node (MLN) of the mutants was increased, and this was consistent with decreased tingible body macrophages (i.e., activated macrophages) in MLN. Splenocytes isolated from the mutants secreted increased levels of IL-6, IFNγ, and IL-17.
Conclusions: Whole-body iPLA2β deficiency led to suppressed innate immunity with a defect in cytokine release by KC. The mutant splenocytes displayed increased levels of pro-inflammatory cytokines which may induce autoimmunity. Upon increased apoptosis in vivo, the mutant KC released exaggerated levels of IL-6 which may promote inflammatory response and liver injury. Thus, iPLA2β's ability to regulate hepatic apoptosis leads to altered functions of immune cells, and this may be related to autoimmune diseases such as autoimmune hepatitis.

P0487

DOMINANCE OF HLA-B RESTRICTED VIRUS-SPECIFIC CD8+ T CELL EPITOPES IN CHRONIC HBV INFECTION

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Background and Aims: HBV-specific CD8+ T cells are essential for HBV clearance and are an attractive target for immunotherapeutic intervention. However, the HBV-specific CD8+ T cell repertoire is poorly characterized. Indeed, <10 HLA-B restricted HBV-specific CD8+ T cell epitopes are described to date, although recent findings in the field of HIV and HCV infection clearly demonstrate a dominant role of these epitopes. We therefore aimed to identify novel CD8+ T-cell epitopes in HBV-infection and also address the relative contribution of HLA-A versus HLA-B restricted epitopes.
Methods: 30 patients with chronic HBV (genotype D) and low viremia were screened for HBV-specific CD8+ T-cell responses using overlapping peptides spanning all HBV proteins in a matrix setup in an elispot assay. Positive responses were confirmed by intracellular interferon gamma staining, optimal epitopes were fine-mapped and HLA restriction was determined. In addition, the autologous viral sequences corresponding to the identified epitopes were analyzed in the patients.
Results: 10 out of 30 patients targeted at least one CD8+ T cell epitope (range: 1–10). In total, we identified 27 CD8+ T cell responses. Interestingly, responses targeted core, polymerase and X proteins, but completely spared the surface antigen. None of these responses targeted previously described HBV-specific CD8+ T cell epitopes. To date, we were able to fine-map 10 novel CD8+ T cell epitopes. Importantly, most of these epitopes were restricted by HLA-B alleles (4 x HLA-B*35; 2 x HLA-B*7; 1 x HLA-B*14; 1 x HLA-B*15). In one epitope restricted by HLA-B*35:02 we found evidence for the selection of viral escape mutations.
Conclusions: In our cohort, HBV-specific CD8+ T cell responses are dominantly restricted by HLA-B alleles. These findings indicate that previous studies on HBV-specific immunity mainly focussing e.g. on HLA-A*02 restricted CD8+ T cell epitopes may not necessarily be conferrable to the complete CD8+ T cell repertoire. They also suggest that HLA-B restricted CD8+ T cell epitopes may be important targets for immunotherapy. The absence of T-cell responses located in the surface-Protein implies a dominant role of the HBs antigen in immune regulation and persistence of HBV.

P0488

DISSECTING THE COMPLEXITY OF INTERFERON RESPONSE THAT CROSS TALKS WITH 4E-BP1 IN HEPATITIS E VIRUS INFECTION

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Background and Aims: Interferon (IFN) signaling represents the first line of host innate defense against invading viral pathogens. Recently, Pegylated IFN-α has been successfully used as an off-label drug for treating hepatitis E virus (HEV) infection. The molecular mechanisms, however, at how IFN signaling interact with HEV infection remain undefined, hampering more wide scale implementation of IFN-α therapy for HEV-infected patients. We comprehensively investigate the interplay between IFN signaling and HEV infection.
Methods: Subgenomic replication and full-length HEV and HCV infectious cell culture models were used. Viral kinetics of five HEV patients treated with Pegylated IFN-α were analyzed.
Results: Screening of 36 cytokines revealed that HEV replication is in general insensitive to regulation by such humoral factors, except for IFN-α that exerts moderate anti-HEV effects in vitro (41% inhibition, $P \leq 0.01$, 1000 IU/ml for 72 hrs). Remarkably, IFN-α completely abrogates of HCV replication. This difference of anti-viral activity is reflected in clinical success of IFN-α therapy towards HCV and HEV infection respectively. Whereas chronic HCV patients respond to IFN-α therapy often with a sharp drop in viral load within the first few days upon treatment, only 1 out of 5 chronic HEV patients exhibited a rapid decline in viral load whereas the others displayed only minor changes during the first month of treatment. In vitro, HEV apparently corrupts antiviral IFN responses via attenuating STAT1 phosphorylation and accordingly suppresses IFN-α-induced transcription of interferon stimulated genes (ISGs; 18 genes tested). In apparent agreement for a role of canonical IFN-α signaling in specifically limiting HEV infection, blocking constitutive IFN signaling (absent of exogenous IFN stimulation) by inhibiting JAK1 results in a release of HEV production (an approx. 5-fold increase over JAK1 proficient cells, $P \leq 0.01$) but not HCV infection. Mechanistically, this effect related to constitutive mTOR-dependent 4E-BP1 activation and subsequent transcription of ISGs as supported from experiments involving RNAi gene silencing, lentiviral overexpression approaches as well as experiments in mouse embryonic fibroblasts generated from mice genetically deficient for 4E-BP1.
Conclusions: IFN-α treatment exerts moderate but delayed antiviral activity against HEV infection in experimental models and in patients. 4E-BP1 restricts HEV infection and mediate the anti-HEV effects of IFN-α throuht modulationg ISG induction.

P0489

INTRACELLULAR CRAWLING OF LYMPHOCYTES THROUGH HEPATIC SINUSOIDAL ENDOTHELIAL CELLS – A NOVEL STEP IN THE ADHESION CASCADE?

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Background and Aims: Excessive lymphocyte recruitment into liver tissue is a hallmark of all chronic inflammatory liver diseases. The recruitment of lymphocytes occurs within the low shear environment of hepatic sinusoids, which are lined by highly specialised endothelial cells (HSEC). We have previously described transcellular migration through HSEC mediated by atypical adhesion molecules (CLEVER-1 and VAP-1). Our aims were to study the underlying mechanisms of this process to identify new targets to treat inflammatory liver disease.
Methods: Using confocal microscopy and flow-based adhesion assays, we compared lymphocyte migration across primary human HSEC and human umbilical vein endothelial cell (HUVEC) monolayers under physiologically relevant shear stress. We also analysed and quantified the junctional molecule expression of these cells in vitro by immunofluorescence and cell-based ELISA. Chemokine expression and monolayer transcellular permeability were also assessed by qPCR and the FITC-dextran assay, respectively. Immunofluorescent staining of human liver tissue was performed to study lymphocyte-endothelium interactions within the hepatic sinusoids.
Results: Live cell imaging of lymphocyte recruitment under conditions of shear stress identified inflammatory cells that

migrated into HSEC and subsequently exhibited novel cell-to-cell crawling. This process was dependent on IFN-γ and occurred predominantly in HSEC when compared to a more conventional vascular endothelium [human umbilical vein endothelial cells (HUVEC)]. Expression of chemokine transcripts and transcellular permeability were similar in both endothelia, whereas distinct differences were identified in junctional molecule expression, with HSEC lacking occludin and expressing lower levels of JAM-A than vascular endothelium. In support of these findings immunofluorescent analysis of human liver tissue demonstrated CD3+/CD4+ cells within sinusoidal endothelial cells.
Conclusions: We demonstrate a novel behaviour of lymphocyte migration which occurs during interactions with primary human HSEC. This process appears to be mediated by the unique junctional expression pattern of HSEC and microenvironmental stimuli such as IFN-γ. We believe this is a novel step in the adhesion cascade that could have implications for treating chronic inflammatory liver disease and modulating intrahepatic immunity.

P0490
NK CELL DYNAMICS IN CHRONIC HEPATITIS B PATIENTS ARE ASSOCIATED WITH HBsAg CLEARANCE AFTER COMBINATION TREATMENT WITH PEGINTERFERON ALFA-2A AND ADEFOVIR
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Background and Aims: The role of natural killer (NK) cells in the clearance of HBsAg is not well understood. Furthermore, it is unknown whether the NK cell phenotype could predict treatment outcome in patients with chronic hepatitis B.
Methods: In a prospective study, 92 (44 HBeAg pos, 48 HBeAg neg) chronic hepatitis B (CHB) patients (HBV DNA >17,182 IU/ml) were treated with peginterferon alfa-2a and adefovir for 48 weeks. PBMC's were collected at baseline, during therapy and follow-up. Response was defined as HBsAg loss at week 72 (functional cure). From this cohort NK cell characteristics of 7 responders (3 HBeAg pos, 4 HBeAg neg) and 7 matched non-responders were analysed, together with 7 healthy controls (HC). Markers of NK cell activation, proliferation, migration and functionality were measured by flowcytometry. Subsequently, 35/44 baseline (BL) samples of HBeAg-positive CHB patients were analysed.
Results: At BL, the NK cell subset distribution ($CD56^{bright}$ and $CD56^{dim}$) of CHB patients was comparable to HC. At end of treatment (EoT), the percentage – as well as absolute numbers – of $CD56^{bright}$ NK cells had increased significantly (11.3% at BL to 44.4% at EoT, $p < 0.0001$), whereas $CD56^{dim}$ NK cells had decreased. At BL, patients with HBsAg loss had significantly lower expression of chemokine receptor CX_3CR1 on $CD56^{bright}$ and inhibitory receptor NKG2A on $CD56^{dim}$ NK cells compared to non-responders (gMFI CX_3CR1 149.7 vs. 222.1, $p < 0.05$ and $NKG2A^+$ 37.3% vs. 50.9% $p < 0.05$). At end of treatment $CD56^{bright}$ TRAIL expression and total NK cell IFNy production was higher in responders compared to non-responders. These differences at BL were not found in BL samples from HBeAg-positive patients with HBeAg seroconversion, but without HBsAg loss (CX_3CR1 $CD56^{bright}$ $p = 0.89$ and $NKG2ACD56^{dim}$ $p = 0.85$).
Conclusions: Baseline expression of inhibitory receptor NKG2A on $CD56^{dim}$ and chemokine receptor CX_3CR1 on $CD56^{bright}$ NK cells are significantly different in CHB patients with HBsAg loss upon peginterferon/ADF combination therapy compared to non-responders. This suggests that NK cells may play a role in the clearance of HBsAg upon peginterferon based therapy.

P0491
IDENTIFICATION OF CD8+ T-CELL EPITOPES SPECIFIC FOR HEPATITIS C VIRUS GENOTYPE 4
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Background and Aims: Virus-specific CD8+ T-cell responses play an important role in the outcome of hepatitis C virus (HCV) infection. Thus, the identification of CD8+ T-cell epitopes is essential for the development of successful vaccination strategies against HCV. To date, most HCV-specific CD8+ T-cell epitopes have been defined in HCV genotype 1 infection. However, in some countries with the highest prevalence of HCV infection, such as Egypt, most patients are infected with HCV genotype 4. Currently, no HCV genotype 4 specific CD8+ T-cell epitopes have been identified.
Methods: In this study, we compared recognition of 93 well described HCV genotype 1-specific CD8+ T-cell epitopes in 20 patients chronically infected with HCV genotype 1 and 14 patients chronically infected with genotype 4. Next, we predicted 40 HCV genotype 4-specific CD8+ T-cell epitopes restricted by 8 different HLA class I alleles most commonly expressed in our genotype 4-infected patient cohort. These epitope candidates were tested for recognition in genotype 4-infected patients.
Results: Only 30% of genotype 1 CD8+ T-cell epitopes are conserved in genotype 4. Indeed, 70% of the 93 genotype 1-specific T-cell epitopes had at least one sequence variation in genotype 4. We were able to detect CD8+ T-cell responses in the majority of genotype 1-infected patients using this set of epitope peptides. However, no CD8+ T-cell responses to these genotype 1-derived epitopes were observed in patients infected with genotype 4. Importantly, we were able to confirm six novel CD8+ T-cell epitopes in genotype 4 infection, restricted by 4 different HLA alleles that are frequently expressed in HCV genotype 4-infected patients.
Conclusions: Epitopes previously identified in HCV genotype 1 infection are not commonly targeted in patients infected with HCV genotype 4. Importantly, we were able to identify the six first HCV genotype 4-specific CD8+ T-cell epitopes. These epitopes may be important targets for vaccination strategies in countries with high prevalence of HCV and dominance of HCV genotype 4 infection. These results further indicate that despite the similarity of HCV genotypes 1 and 4 in respect to antiviral therapy, T-cell based vaccines need to be adapted to the respective genotype.

P0492
ASSOCIATION OF CYCLIN D3 GENE PROMOTER HYPERMETHYLATION WITH HEPATITIS B e ANTIGEN POSITIVE STATUS AND INCREASED VIRAL REPLICATION IN HEPATITIS B VIRUS INFECTED PATIENTS
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Background and Aims: Approximately 400 million people are chronically infected with Hepatitis B virus (HBV) worldwide. HBV integrates into human genome and triggers an innate methylation response that inactivates the viral inserts but also methylates adjacent host DNA. If the tumour suppressor and other cell-regulating genes are affected, these epigenetic changes could cause hepatic inflammation, fibrosis and HBV-induced hepatocellular carcinoma (HCC). We have previously shown that many gene promoters, including Cyclin D3 (CCND3) are hypermethylated in HBV patients. CCND3 gene regulates the cell cycle through

interaction with tumour suppressor genes. High CCND3 protein expression is present in cirrhotic livers with HBV-induced HCC, suggesting an important role in HCC development. We investigated whether CCND3 gene promoter hypermethylation was associated with hepatitis B e antigen (HBeAg) status and viral replication.
Methods: Bisulfite DNA sequencing technique was used to analyse CCND3 gene promoter CpG islands for the presence of hypermethylation in HBV-infected patients and normal controls. DNA samples were extracted from 30 individuals; 10 HBeAg positive, 10 HBeAg negative and 10 normal controls. Bisulfite converted DNA was cloned, sequenced and then analysed with BioEdit Sequence Alignment Editor to create ClustalW multiple alignment of the CCND3 gene promoter sequence extracted from Ensembl human genome 19 assembly with the bisulfite converted sequence of positive clones. The demographic and clinical data of the study patients was analysed using STATA 13.
Results: CCND3 gene promoter hypermethylation was significantly higher in HBeAg positive (80%) compared to HBeAg negative (40%) individuals ($p = 0.001$). No methylation was detected in normal controls. HBeAg positive patients had significantly higher DNA levels ($p = 0.02$) compared to HBeAg negative patients.
Conclusions: CCND3 gene promoter hypermethylation was associated with HBeAg positive status and high viral load. The high viral load most likely leads to a higher rate of HBV genome integration and therefore methylation. Patients with sustained viral replication have more epigenetic changes. This may explain the documented association of cirrhosis and HCC with high viral load and HBeAg status. However, the role of genotype, type and position of HBV inserts in triggering methylation needs further investigation with larger cohort.

P0493
SELECTIN-TARGETING NANOPARTICLES FOR IMMUNOMODULATORY THERAPY OF LIVER DISEASES
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Background and Aims: Selectins are cell adhesion mediating molecules, which specifically facilitate leukocyte recruitment to various tissues. Specific inhibition of L, E, or P selectin receptors using artificial ligands may represent a novel strategy to manipulate immune cell behavior for ameliorating inflammatory liver disease.
Methods: We synthesized four different fluorescently labelled polymer-carbohydrate conjugates P1-P4 (Dh=10 nm) with a poly N-(2-hydroxypropyl)methacrylamide (pHPMA) backbone. The conjugates can be divided into 2 classes, SLe^x-pHPMA conjugates [non-sulfonated (P1) and sulfonated (P2)] and mimetics thereof [non-sulfonated (P3) and sulfonated (P4)], which present the single carbohydrates of the natural Ligand SLe^x involved in selectin binding randomly among the polymer back bone. Mimetics are promising since they reduce the 51-step synthesis of SLe^x-pHPMA conjugates dramatically. Binding to different selectin receptors was assessed via surface plasmon resonance. We isolated different human primary cells, and studied their interactions with the constructs *in vitro* using cell culture (mRNA expression of inflammatory mediators and migration assays) and flow cytometry (particle interaction studies). Ultimately, the biodistribution and usability of the polymer-carbohydrate conjugates as nanotherapeutics were assessed *in vivo* in healthy C57BL/6J mice as well as in a model of acute liver injury based on carbon tetrachloride (CCl_4).
Results: The constructs bound with different intensities to distinct human primary cells *in vitro* as assessed using flow cytometry. P2 and P4, if added to macrophage cultures prior to treatment with lipopolysaccharides (LPS) led to a significantly increased expression of TNF *in vitro*, and P2 also significantly stimulated CCL2 production by human macrophages, compared to LPS alone. P3 significantly inhibited macrophage migration *in vitro*. In homeostasis, all constructs accumulated in hepatic macrophages, and P3 most clearly accumulated in liver. In CCl_4-based acute liver injury, P1 significantly increased whereas P3 significantly attenuated liver injury reflected by histology and transaminase levels. P4 exhibited no significant effects on liver injury.
Conclusions: Selectin-directed conjugates appear to exhibit a strong potential as immunomodulatory agents for the treatment of liver diseases based on their effects on macrophage migration and activation as evidenced *in vitro* and *in vivo*.

P0494
NATURAL KILLER AND NATURAL KILLER T CELLS FROM IMMUNE ACTIVE HEPATITIS B e ANTIGEN NEGATIVE PATIENTS EFFECTIVELY INDUCE APOPTOSIS AND SUPPRESS COLLAGEN PRODUCTION IN HEPATIC STELLATE CELLS
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Background and Aims: Natural killer (NK) cells have been shown to attenuate hepatic fibrosis in transgenic HBV murine models. There is a paucity of data regarding the role of human NK and NKT cells in hepatitis B virus associated fibrogenesis. The aim of this study was to investigate the potential anti-fibrotic effects of human NK and or NKT cells from immune active HBV patients on HSC.
Methods: Immune active (IA) HBV was defined as ALT ≥5 ULN and Immune control (IC) was defined as normal ALT. Human NK (90–96% purity) and NKT cells (75–85% purity) were magnetically isolated from peripheral blood mononuclear cells and cultured with LX2 human hepatic stellate cells (HSC). NK and NKT cells were further phenotyped by Tim-3, NKG2A, NKG2D, NKp46, FasL and TRAIL expression. Induction of LX2 apoptosis was analysed by caspase-3 activation. Intracellular cytokine production (IFN-α and TNF-α) and CD107a degranulation were used to determine NK and NKT cell functional activity. HSC collagen production was determined by the Sircol assay.
Results: 15 patients were recruited: 6 IA HBV, 4 IC HBV and 5 healthy controls (HC) [serum ALT (IU/ml): 260.83±232 vs 24.25±8.93 and 24±5.42 respectively, $p = 0.007$]. Median HBV DNA viral load was higher in IA compared to IC (6.29 log10 IU/ml, IQR: 4.8–7.49 vs 2.26 log10 IU/ml, IQR: 1.73–2.88; $p = 0.019$). NK and NKT cells from IA HBV were significantly more effective in HSC apoptosis induction than similar cells from IC HBV or HC (16.15% ± 13.8 vs 5.17% ± 2.31 and 2.67% ± 0.85, respectively; $p < 0.0001$). HSC caspase-3 expression correlated with serum ALT [Spearman's rho (ρ) = 0.66, $p < 0.0001$], reduced proline incorporation ($\rho = -0.62$, $p < 0.0001$) and increased NKp46 expression on NK^{Bright} cells ($\rho = 0.56$, $p = 0.04$) and NKG2D expression on NKT cells ($\rho = 0.54$, $p = 0.03$). There was no significant difference in cytokine production, TRAIL or FasL expression.
Conclusions: NK and NKT cells from immune active HBeAg negative patients effectively induce HSC apoptosis, which is associated with reduced collagen production. NK and NKT cells may exert an anti-fibrotic effect during the immune active phase of CHB through upregulation of the activating receptors NKp46 and NKG2D.

P0495

PATHOLOGICAL LIVER CONDITIONS ABROGATE INTRAHEPATIC MYELOID CELL TOLERANCE TO BACTERIAL PRODUCTS

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Background and Aims: Despite their tolerogenic properties, intrahepatic myeloid cells (macrophages and monocyte-derived macrophages) are an important defensive barrier against bacteria dissemination and play a role in chronic liver injury. Whether this functional heterogeneity is altered by pathological liver conditions has not been analyzed in humans. We therefore studied the impact of pathological liver conditions on the composition and function of human liver-resident myeloid cells.
Methods: Intrahepatic myeloid cells were purified from cold perfusates of healthy (living donor liver transplants, n = 8) and pathological (HBV- or HCV-related cirrhosis n = 6) livers. Their phenotypic composition, immune gene profile and functional ability to produce pro-inflammatory, suppressive, antiviral and pro-angiogenic factors *ex vivo* and after activation with live bacteria and TLR agonists were analyzed.
Results: Despite a similar phenotypic composition, the immune gene profile of myeloid cells (CD14+) purified from healthy livers differs from that of the peripheral blood. We detected a constitutively low pro-inflammatory (IL-1b, IL-6, TNF-alpha, PLAU, PLAUR and PTGS2) immune gene expression in healthy intrahepatic myeloid cells compared to those in peripheral blood. Healthy intrahepatic myeloid cells trigger a robust antibacterial response (activation of MAIT and NK-bright cells through IL-12 and IL-18 production) when stimulated with whole bacteria and TLR-8 agonist (ssRNA40) but are hyporesponsive to TLR agonists (TLR-1/2, 2, 2/6, 4 and5) that mimic bacterial products. Pathological livers are instead enriched in (HLA-DRhi CX_3CR1hi) CD14+ CD16+ intermediate monocytes and contain myeloid cells which express a classical inflammatory gene profile producing high levels of pro-inflammatory cytokines (IL-1b, IL-6 and TNF-alpha), chemokines (CCL-3 and CCL-4) and angiogenic factors (VEGF) directly *ex vivo* and after activation with bacterial TLR ligands.
Conclusions: Pathological liver conditions shape the composition and function of intrahepatic myeloid cells. Myeloid cells from healthy human livers trigger an efficient immune response against whole bacteria but are tolerant to bacterial products. This functional profile is reversed in myeloid cells during pathological conditions. These results suggest that translocation of bacterial products from the gut can sustain liver inflammation in pathological livers.

P0496

IMPAIRED NEUTROPHIL FUNCTION CONTRIBUTES TO LIVER INJURY AND POSITIVELY CORRELATES WITH CLINICAL SEVERITY INDICES IN ACUTE-ON-CHRONIC LIVER FAILURE

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Background and Aims: Impaired neutrophil function has been demonstrated in acute liver failure and serves as a biomarker involved in organ dysfunction. However, its role in acute-on-chronic liver failure (ACLF) is not known. We assessed functional alterations of neutrophils associated with hepatic injury in 17 hepatitis B virus-related ACLF (HBV-ACLF), 19 alcohol-related ACLF (alcoholic-ACLF), and 42 chronic hepatitis B (CHB) patients in comparison to 18 healthy controls (HC).
Methods: Neutrophil phagocytic activity (NPA) was determined by uptake of opsonized *Escherichia coli* and reactive oxygen species (ROS) with or without *E. coli* stimulation. CXCR-1 and CXCR-2 expression was analyzed by flowcytometry, RT-PCR and immunohistochemistry. Role of neutrophils in hepatic injury was determined by co-culturing of LPS stimulated neutrophils or their supernatant with HepG2 cells.
Results: Neutrophils percentage and absolute neutrophil count (ANC) was significantly higher in both HBV and alcoholic-ACLF patients than CHB and HC. Importantly, ACLF patients having MELD score more than 30 had higher ANC ($p < 0.04$) than those with lower MELD score. In ACLF, NPA was highly impaired ($p < 0.02$, 0.007) and significantly associated with MELD ($p < 0.04$) and CTP score ($p < 0.007$). ROS was also increased ($p < 0.05$, 0.005) and showed positive correlation with inflammatory cytokines, IL-6 ($p < 0.05$), IL-17 ($p < 0.05$) and CXCL-8 ($p < 0.008$). Significant up-regulated CXCR-1 and 2 expressions were seen in ACLF; more so near necrotic areas of liver tissue revealing more infiltration of neutrophils on sites of injury in ACLF. CXCR-1 and 2 expressing neutrophils from ACLF patients significantly induced early apoptosis and necrosis of HepG2 cells by direct contact and cytokine/ROS dependent mechanisms. Further we also found higher gene expression of apoptosis (caspase-3 $p < 0.02$, 0.008) and necrosis marker (receptor interacting protein-3 $p < 0.008$, 0.003) in the liver of ACLF patients which may indicate neutrophil induced cell death in these patients. ACLF also had significant increased inflammatory cytokines (IL-6, IL-17, IL-23, CXCL-8, CCL-20 and GM-CSF).
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Conclusions: ACLF patients have increased neutrophils with high CXCR1/CXCR2. These neutrophils have impaired phagocytic activity with high ROS and pro-inflammatory cytokines propagating hepatic injury by necrosis/apoptosis which may further induce organ dysfunction. Neutrophil markers present a powerful tool for drug targets and clinical management of ACLF.

P0497

SECRETORY LEUKOCYTE PROTEASE INHIBITOR (SLPI) SUPPRESSES INNATE IMMUNE RESPONSES AND PROMOTES RESOLUTION OF INFLAMMATION IN AN AUTO/PARACRINE MANNER DURING ACUTE LIVER FAILURE (ALF)

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Background and Aims: Monocytes/macrophages (Mo/h-Mφ) orchestrate resolution responses during acute liver failure (ALF). We recently identified Secretory Leukocyte Protease Inhibitor (SLPI) as a pivotal mediator that suppresses NF-kB-dependent pro-inflammatory responses in ALF. Our aim was to determine the effects of SLPI on both innate immune and resolution responses in human ALF.
Methods: Immunophenotyping of Mo/h-Mφ from liver explants was performed by flow cytometry using a panel of 15 surface markers (n = 30). Immunohistochemistry/multispectral imaging analysis (# cells/high power field [HPF]) was used to examine the h-Mφ phenotype in ALF/control tissue (n = 10). Effects of recombinant human (rh)-SLPI (0.5 ug/ml) on Mo/h-Mφ, neutrophil and NK cell phenotype, function (LPS-stimulated cytokine secretion, uptake of apoptotic neutrophils/hepatocytes; n = 5) and migration across hepatic sinusoidal endothelial cells (HSECs) (n = 3), were examined using confocal microscopy, flow cytometry and ELISA.

Neutrophil survival and LPS-stimulated cytokine secretion were determined in culture with (rh)-SLPI and anti-SLPI neutralizing antibody in healthy control/ALF Mo-derived supernatants (n = 10).
Results: Compared to normal and cirrhotic liver tissue, h-Mφ in ALF exhibit a pro-resolution phenotype (HLA-DRlowCD163highMerTK++) [Fig. A], detected within areas of hepatic necrosis (34vs428 CD163+MerTK+ cells/10HPF; p = 0.0002). Whilst neutrophil and NK cell survival and pro-inflammatory cytokine secretion were not modulated directly by (rh)-SLPI administration, SLPI induced a pro-resolution phenotype in Mo/h-Mφ (CD163high MerTKhigh CCR5low) [Fig. B], which were functionally characterised by: (i) decreased pro-inflammatory cytokine secretion (TNF-α, IL-6, IL-8 and IFN-γ; all p < 0.05), (ii) reduced Mo recruitment across HSECs [Fig. C(i)] and (iii) enhanced uptake of apoptotic neutrophils and hepatocytes [Fig. D]. Importantly, neutrophils cultured in ALF Mo-derived supernatants [Fig. E] showed reduced survival (26.15% vs 21.3% apoptotic cells) and levels of pro-inflammatory cytokine production [TNF-a; 9.8% vs 14.2% cells], which was reversed by inhibiting the activity of SLPI (1 ug/ml a-SLPI).
Conclusions: We reveal that SLPI is a pivotal mediator of resolution responses in ALF through an autocrine negative feedback loop in addition to paracrine suppression of neutrophil survival and pro-inflammatory responses, thereby identifying a novel mechanism of innate immune suppression in ALF.

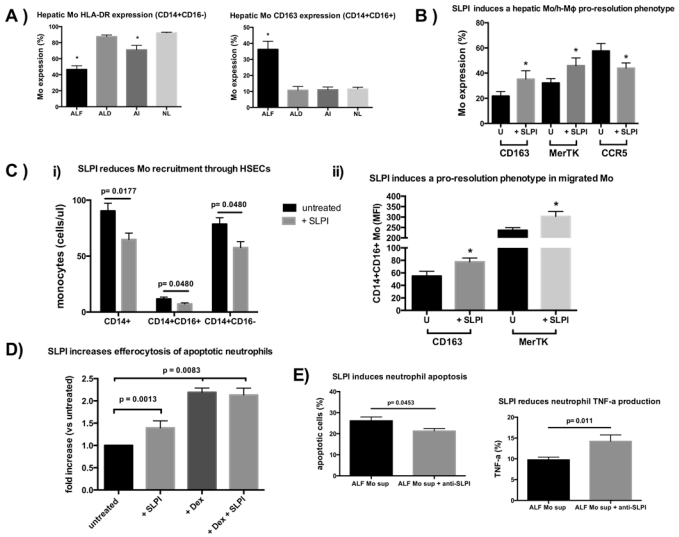

Figure: Secretory Leukocyte Protease Inhibitor (SLPI) suppresses innate immune responses and promotes resolution of inflammation in human acute liver failure (ALF). (A) *Ex vivo* HLA-DR and CD163 expression of hepatic Mo/h-Mφ [acute liver failure (ALF), alcoholic (ALD), auto immune (AI) liver disease and resections (NL)]. Effects of rh-SLPI (0.5 ug/ml) on: (B) hepatic Mo/h-Mφ phenotype (48 h), (C) monocyte migration through HSECs (i) number of migrated monocytes through HSECs/collagen and (ii) CD163 and MerTK migrated monocyte expression, (D) monocyte uptake (6 h) of apoptotic neutrophils after healthy monocyte culture with SLPI and 100nM dexamethasone (48 h). (E) neutrophil survival and TNF-a production of healthy neutrophils cultured in ALF plasma-treated monocyte supernatants +/− anti-SLPI neutralizing antibody (6 h). [*p < 0.05]

P0498

DEFINING TISSUE-RESIDENT CD8 T CELLS IN HUMAN LIVERS

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Background and Aims: Tissue resident T cells that behave more like innate rather than classical adaptive effectors cells can provide a rapid immune response upon pathogen encounter, and have been observed in murine skin, lung and intestinal/vaginal mucosa. Our aim was to determine whether a liver resident T cell population with peculiar functional features exists in human livers.
Methods: We performed a comprehensive analysis of immune genes expression, phenotype and function of CD8 T cells obtained from the liver and blood of healthy human donors using Nanostring, flow cytometry and multiplex bead array respectively.
Results: CD8 T cells in the liver have a profile of human immunology gene expression that is distinctly different from their peripheral blood counterparts. They express lower levels of SELL (CD62L) and CCR7, and higher levels of ITGAL (CD11a) and CD69 than blood CD8 T cells, characteristic of tissue-resident memory T cells. Unlike the resting CD8 T cells found in the peripheral blood, these liver-resident CD8 T cells have a reduced TH1 cytokine production, but higher expression of mRNA coding for cytotoxic granules (granzyme B and perforin) upon TCR engagement. Furthermore, they express high levels of STAT4, comparable to that found in effector cells sensitive to cytokine-mediated activation.
Conclusions: We demonstrated the presence of CD8 T cells in human livers with phenotypical and functional features unique to the organ. Their ability to respond to defined cytokines is under investigation.

P0499

FUNCTIONAL ANALYSIS OF GENETIC VARIANTS OF TOLL-LIKE RECEPTOR (TLR) 9 AND INTEFERON REGULATORY FACTOR (IRF) 7 ASSOCIATED WITH THE NATURAL COURSE OF HCV INFECTION

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Background and Aims: Toll-like receptors (TLR) are the main components of the innate immune system and form the first defence line during hepatitis virus C (HCV) infection. The TLRs recognize specific motifs within the virus and trigger downstream via myeloid differentiation primary response gene 88 (MyD88)-dependent signalling cascades to activate the production of interferons, cytokines and chemokines. HCV RNA is usually detected by the intracellular TLR3 and TLR7. TLR9 seems to take part in HCV recognition, although viral RNA is no typical substrate of this receptor. Previously, we were able to show associations of polymorphisms (SNPs) within the promoter of the TLR9 gene and within the interferon regulatory factor (IRF) 7 gene with spontaneous viral clearance and with the severity of liver disease. In this study we investigated the functional impact of those SNPs on the signalling pathway in cell culture.
Methods: Reporter constructs of TLR9 gene (wild type, −1486/C and −1237T/C) and expression plasmids of IRF7 protein (wild type and K192E variant) were generated. The TLR9 plasmids were transfected in Nawalma R20 B-cell culture and the constitutive and stimulated TLR9 promoter activity was measured via the Dual-Luciferase reporter assay. Analysis of the interaction of the IRF7 plasmids with possible partners was performed via LUMIER method in HEK293T cells.

Results: In Nawalma R20 B-cells significant differences in basal promoter activity were observed between TLR9 wild type promoter and the genetic variants. The homozygous CC genotypes of the two SNPs showed higher activities (−1486T/C $p < 0.01$; −1237T/C $p < 0.001$) than the wild type. The presence of homozygous minor variants in both SNPs was associated with a significant reduction of promoter activity as compared to the activity observed in carriers of a single SNP. Stimulation of the TLR9 promoter with CpG ligands suppressed the activity independent of genotype. LUMIER analysis revealed significantly weaker binding of the IRF7 K192E variant with MyD88, IRAK1, TRAF6 and TBK1.
Conclusions: Variants of the TLR9 which are associated with spontaneous HCV clearance showed higher promoter activity and may contribute to enhanced induction of type 1 interferons and pro-inflammatory cytokines. In contrast, the IRF7 K192E variation may promote HCV chronicity due to an impaired binding to partners of the MyD88-depending signalling cascade.

P0500

HIV AND HEPATITIS DELTA – THE LAST CHALLENGE IN THE VIRAL HEPATITIDES?

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Background and Aims: Liver disease in HIV remains a significant clinical issue, predominantly related to BBV. Hepatitis Delta (HDV) is under-ascertained with little data on clinical outcomes. The aim of this cohort study was to compare the clinical outcomes of HDV in HIV-positive HDV with HIV-negative HDV in an ethnically diverse South London environment.
Methods: Retrospective data on pts who had a positive HDV total Ab test (HDV IgG) from Jan 2000 to Aug 2014 were collated. Pts referred for transplant assessment or HCC were excluded as not part of our natural cohort. A cohort of HIV-positive HDV pts from another London HIV centre were included.
Results: 241 pts had positive HDV Ab. 50 were excluded from the analysis as they were tertiary referrals. Of the 191 remaining pts, 17 were HIV positive, 112 HIV negative, 62 HIV status not documented. 90 patients had cleared HDV at the time diagnosis. This was defined by HDV IgM and RNA negativity or only HDV IgM negative alone if pre 2009. 4/17 HIV-positive pts cleared HDV at baseline, 66/112 HIV-negative pts cleared HDV at baseline ($p = 0.009$). 8 pts with HIV-positive HDV from another clinic were included. 2 had evidence of active HDV infection, giving a total of 15 HIV-positive pts with active HDV.
The analysis focuses on the 15 HIV-positive HDV pts and the 46 HIV-negative HDV pts. HIV-positive patients were 80% male, 53% black African, HIV-negative pts were 59% male, 58% black African.
The first available median HDV RNA levels were higher in HIV-positives, 8.3E5 cps/ml (6.6E5–9E6) compared to 2.3E4 (1.9E3–6.8E5) in HIV-negatives ($p = 0.04$).
Of HIV-positive HDV pts, 4/17 were treated with peg-interferon, 1 cleared HDV after 2× therapy, 2 relapsed, 1 discontinued treatment and was lost to FU. Of HIV-negative HDV pts, 15 were treated, 8 cleared HDV, 4 non response, 2 relapsed, 1 stopped.
8/15 HIV-positive HDV pts had cirrhosis at diagnosis vs 6/46 HIV-negative HDV pts ($p = 0.003$). Of the 8/15 HIV-positive HDV cirrhotic pts, 2 died of liver causes, 2 decompensated, 2 were lost to FU. Of the 6/46 HIV-negative HDV cirrhotic pts, there was 1 decompensation and 2 liver deaths. Using a composite endpoint of death, decompensation and cirrhosis, HIV was the only factor associated in univariate analysis ($p = 0.002$); age, risk factor, HDV RNA level and AST were not. With a composite endpoint of death or decompensation, HIV, risk factors and age were all significant but in the final model, only HIV status was predictive ($p = 0.006$).
Conclusions: Compared to HIV-negative HDV pts, those with HIV-positive HDV infection were less likely to spontaneously clear HDV. Patients with HIV-positive HDV suffered significant morbidity and mortality. Better ascertainment and therapies for HDV are urgently needed.

P0501

REDUCED T CELL ACTIVATION, REGULATORY T CELL MEDIATED SUPPRESSION AND COMPROMISED NATURAL KILLER CELL FUNCTION CHARACTERIZE IMMUNE TOLERANT PHASE OF CHRONIC HBV INFECTION

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Background and Aims: Chronic HBV infection (CHI) acquired perinatally or early in life and typified by HBeAg positivity, high viral replication, but normal serum aminotransferases and minimal/no liver inflammation represent the Immune-tolerant (IT) phase of CHI. We investigated potential roles of various arms of host immune system that contribute towards immune tolerance as compared to their functions during chronic Immune-active hepatitis (IA).
Methods: Phenotype and function of $CD8^+$T cell, natural killer (NK) cells and classical regulatory T (Treg) cells was evaluated using standard flow cytometric assays in IT and IA patients and healthy controls.
Results: T cells were similar in percentages in IT and IA but varied significantly in phenotype and function. IT, unlike IA, had significantly higher $CD28^+$T cells but lower PD-1^+T cells denoting absence of T cell exhaustion. While a large fraction of circulating T cells from IT expressed CD69, suggestive of early immune activation, significantly lower numbers of T cells expressing late activation marker HLADR was noted that depict ineffectively primed and inconsistent T cell response. Hence, T cells in IT although transiently activated could not maintain sustained activation status, consequently delaying viral clearance and simultaneously restraining tissue damage. Moreover, memory $CD45RO^+$T cells were greatly decreased in IT that may negatively impact recall response on antigen reencounter. However, upon invitro stimulation, global and HBV specific T cells of IT, retained greater potential to produce IL-2 and IFN-γ suggesting that T cells in IT are capable of mounting antiviral response following adequate stimulation. Conversely, NK cells were quantitatively and qualitatively defective in IT with significantly lower incidence of NK cells expressing NKG2C, NKp44, TRAIL, Perforin, CD107a as well as IFN-γ. Surprisingly, both groups displayed comparable Treg suppressive potential as appraised by similar expression pattern of CTLA-4, CD39, FOXP3, TGF-β and IL-10. Thus reduced ability of NK cells to release IFN-γ and enhanced IL-10 secretion by Treg cells seems to hinder prolonged T cell activation in IT.
Conclusions: Collectively, our data imply that lesser magnitude of T cell activation, smaller memory T cell pool along with enhanced Treg activity and compromised NK cell function limit immunological reactivity to high levels of HBV during IT phase.

P0502

RECOMBINANT NEUROLIGIN 4 (NLG4) ACTIVATES NK CELLS AND ATTENUATES FIBROGENESIS IN NONALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Among the anti-fibrotic responses NK cells induce hepatic stellate cell (HSCs) apoptosis (J Hep 2004). NK cell lose their killing effects in advanced fibrosis and cirrhosis (Gut 2011). NK cells from nonalcoholic fatty liver disease (NAFLD) patients exert high expressions of neuroligin-4 receptors (NLG4) and are thought to interfere with NK functionality (AASLD 2011 and EASL 2013). NLG4 receptor immune synapse interactions with its ligand β-neuroxin (cell-adhesion molecules) are thought to play an important step in fibrogenesis. We aimed to develop NK activation tool to ehance anti-fibrotic effects via NLG4 pathway in an *in vitro* co-culture condition.
Methods: NK cells isolated form peripheral blood of NAFLD patients lacking metabolic syndrome were incubated with 10 nM of recombinant NLG4 for 3 hours prior to incubations with HSCs; LX2- cell line. Following 24 hours, cells were trypsinized and analyzed by flow-cytometry for NK activity by CD107a [Lysosomal-associated membrane protein 1 (LAMP1) – degranulation marker – a marker for NK activation] and LX2 activities (a-smooth-muscle intensities). Interleukin-4 (IL4) quantity expressions were also assessed in NK cells.
Results: LX2 cell line express 75% of β-neuroxin. In co-cultures, recombinant NLG4 significantly increased lysosomal-associated membrane protein-1 (CD107a, NK activation marker) from 10% to 48% ($P < 0.01$). Compared to LX2 mono-cultures, elevations of NK cells CD107a activity was associated with increased LX2 killing; as aSMA mean fluorescence intensities of HSCs decreased from 3938 in cultures without the recombinant to 2432 with the recombinant ($P < 0.01$). NK cells pre-treated with the recombinant NLG4 showed decreased in IL-4 secretions by the flow cytometry technique (1.8-folds, $P = 0.02$).
Conclusions: NLG4-β-neurexin recognition mediates HSCs-NK immune synapse to control release of NK vesicles. Recombinant NLG4 activates NK cells to promote anti-fibrotic effects through increased HSCs killing. These effects were associated with decreased expression of the pro-fibrogenic marker IL4 in the NAFLD NK cells. NLG4 immune modulation of CD107a activity of NK cells extends the understandment and therapeutic strategies in fatty liver disease.

P0503

DENDRITIC CELL SUBSETS AND EXPRESSION OF DC-SIGN IN PROGRESSION OF HEPATITIS B INFECTION FROM ACUTE TO CHRONIC INFECTION: A NOVEL INSIGHT THROUGH TLRS AND RELATED INTRACELLULAR SIGNALLING MOLECULES

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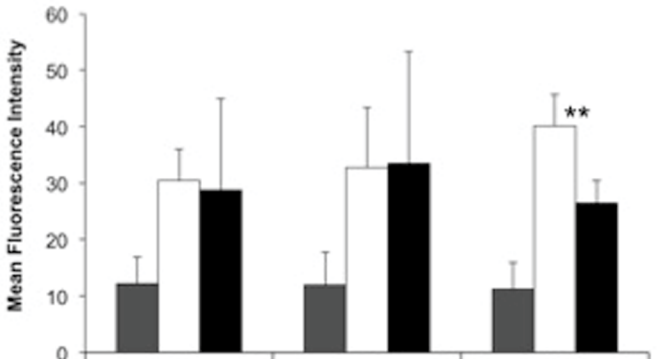
Background and Aims: DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DCSIGN) and Toll-like receptors (TLRs) which are pathogen recognition receptors (PRRs), present on Dendritic cells (DC) to promote pathogen recognition, uptake and presentation of antigen and potentiates the interaction with T cells. We hypothesize, insufficient generation of immune surveillance in chronic hepatitis B might be because of impairment of frequencies and functional ability of DC's and PRR's. We aimed to monitor temporal changes in DC's during acute phase in comparison to chronic hepatitis B infection.
Methods: Twenty-eight subjects were included; acute viral hepatitis B (AVH-B, n = 8) were serially followed up; chronic hepatitis B (CHB, n = 10) and healthy controls (HC, n = 10). PBMCs were immunostained before and after stimulating with polyI:C, CpGODN and LPS with flourochrome labelled antibodies. The frequency of myeloid DC's (mDC) (CD11c+, BDCA1+), plasmacytoid DC's (pDC's) (CD123+, BDCA1+), intracellular IFN-γ production and expression of DCSIGN, TLR2,3,4 and TLR9 was evaluated. mRNA levels of adaptor molecules of TLR pathway; Myd88, TRAF6 and cytokines IL-2, IL-6, TNF-α, IFN-α was measured by RT-PCR.
Results: Frequencies of both subsets of DC's i.e. mDC's and pDC's, were found significantly higher in acute and naïve chronic HBV patients than healthy controls ($p < 0.00$, $p < 0.01$) but no difference among AVH-B vs CHB. Functionally impaired DC's were seen with significant low IFN-γ production and low DCSIGN expression ($p < 0.04$, 0.00) in CHB patients. Even after stimulations by TLR agonist, no changes were found. The adaptor molecule of TLR's, the MyD88 mRNA levels were also downregulated along with the cytokines IL-6, IFN-α. On follow-up of AVH-B patients, found temporal changes in DC's while mDC's frequency was significantly highest at wk 4, whereas pDC's at wk 6, than wk0 ($p < 0.02$, 0.01) along with the DCSIGN on mDC's expression increased linearly from wk 0 to wk 4 during acute phase (wk 0 28% to wk 4 72%, $p < 0.00$).
Conclusions: (i) Subsets of dendritic cells are phenotypically intact but functionally impaired in CHB compared to seroconverted AVH-B patients. (ii) During acute phase pDC's comes late in generation of effective immune response. (iii) DC SIGN expression is temporally increased in acute phase HBV infection, while is significantly low in CHB, indicating its key role in persistent infection. These novel observations would pave way for prognostication and development of antiviral strategies for chronic HBV infection.



	mDC	pDC	IFN-Y
HC	12.2	11.9	11.2
AVHB	30.5	32.8	40.1
CHB	28.7	33.5	26.5

Figure 1. INF-γ production was significantly low in persistent CHB patients. No significant difference was found in frequency of mDC's and pDC's in acute and chronic HBV infection.

P0504

DE NOVO NUCLEOTIDE BIOSYNTHESIS PATHWAY TIGHTLY REGULATES HEPATITIS E VIRUS INFECTION

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Background and Aims: Despite an emerging global health issue, no approved drug is available for treating hepatitis E virus (HEV) infection. Since nucleotides are key components involved in host cell metabolism and virus infection, inhibitors of nucleotide synthesis enzymes may be potential drugs against HEV. Biosynthesis of purine nucleotides requires guanosine, which can

be phosphorylated into GMP, GDP and GTP. Pyrimidine nucleotides synthesis requires uridine, which can be catalyzed into UDP and UTP. We thus investigated the effects and mechanism-of-action of targeting de novo nucleotide synthesis in cell culture models of HEV infection.

Methods: A subgenomic HEV replication model expressing luciferase reporter gene and a full-length infectious model were used.

Results: Supplementation of exogenous guanosine, but not uridine, promoted HEV replication in both subgenomic (2.6-fold) and infectious (2.0-fold) models. We thus focused on sequentially targeting key enzymes involved in purine synthesis pathway. Surprisingly, 6-thiohuanine, an inhibitor of Amido phosphoribosyltransferase (APRTase), stimulated HEV replication by 3.6±0.87 folds (Mean±SEM, n = 10, P < 0.0001). Lometrexol, an inhibitor of GAR Transformylase, also promoted HEV replication in both models. ATIC is a bifunctional protein that exerts AICAR transformylase activity and IMP cyclohydrolase activity in this pathway. Methotrexate (MTX) and Fludarabine (FA) are inhibitors of AICAR transformylase and IMP cyclohydrolase, respectively. Both compounds potently increased HEV genome RNA by 5.01±1.4-fold and 5.06±0.3-fold, respectively (n = 5, P < 0.05). Consistently, silencing of ATIC resulted in dramatic increase of HEV RNA by 4.2±2.5-fold and abolished the ability of MTX to activate HEV. This demonstrated that ATIC has anti-HEV activity, which explains the proviral effects of MTX and FA. However, Mycophenolic acid (MPA), an inhibitor of inosine monophosphate dehydrogenase (IMPDH), could effectively inhibit HEV replication by 65% (10 ug/ml).

Conclusions: Purine but not pyrimidine synthesis pathway regulates HEV infection. Unexpectedly, targeting the early steps of this pathway led to enhancement of HEV infection. Whereas targeting the late step (IMPDH enzyme) resulted in potent antiviral activity against HEV.

P0505

IDENTIFICATION OF IL-17 POSITIVE INTRAHEPATIC NK CELLS IN HUMAN LIVER AND RELATIONSHIP WITH CHRONIC LIVER DISEASES

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Background and Aims: The production of pro-inflammatory IL-17 cytokine plays an important role in many inflammatory diseases and is not restricted to Th17 cells. However, in liver diseases, it has not yet been investigated if intrahepatic (IH)-natural killer (NK) cells, the major subset of innate immune cells, produce IL-17 and thereby contribute to liver inflammation.

Methods: To analyze production of IL-17 by IH-immune cells, we performed flow cytometry analyses of fresh liver biopsies from 54 patients: NASH, HCV and HBV-infected and non-inflammatory subjects. Localization of IL-17$^+$ NK cells was studied by double immunohistochemical and triple immunofluorescence analyses. Frequency of IL-17 producing NK cell was compared with INFγ production and correlated with clinical parameters.

Results: Flow cytometry analyses revealed that, in addition to T lymphocytes, IH-NK and IH-NKT cells produced IL-17 cytokine with frequency from 0.1% to 6.0%, depending on liver environment. The population of IL-17$^+$IH-NK cells was significantly higher compared to IFNγ producing IH-NK cells (p = 0.009) and mainly specific to the NKp46$^+$ NK cells subset (p < 0.001). Immunohistology of livers showed that 85% of IL-17$^+$NKp46$^+$ IH-NK cells were localized in septal or portal fibrous area. Finally, the proportion of IH-NK cells producing IL-17 increased during the critical A2 Metavir necro-inflammatory grade, suggesting a specific role of IL-17$^+$ IH-NK cells in the development of liver inflammation.

Conclusions: Our study provides evidence that in human liver, NK cells produce IL-17$^+$ and thus play an active role in shaping the local inflammatory response, in addition of their cytotoxic and regulatory functions.

P0506

IMPAIRED HCV-SPECIFIC CYTOTOXIC T CELL REACTIVITY DUE TO 4-1BB SIGNALLING ADAPTER (TRAF1) DOWN-REGULATION DURING PERSISTENT HCV INFECTION IS RESTORED BY IL-7 PLUS 4-1BBL TREATMENT

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Background and Aims: During persistent hepatitis C virus (HCV) infection, HCV-specific cytotoxic T cell (CTL) response is impaired and featured by IL-7 receptor (CD127) down-regulation. IL-7 could impact on T cell reactivity by regulating 4-1BB signalling adapter (TRAF1). In this study, TRAF-1 expression on HCV-specific CTL according to viral control and, the role of IL-7 on TRAF-1/4-1BB signalling and CTL reactivity were addressed.

Methods: Peripheral blood mononuclear cells (PBMC) from 6 healthy donors (HD), 12 HLA-A2$^+$ genotype-1 HCV$^+$ sustained viral responders after treatment (SVR) and 16 HLA-A2$^+$ chronic naïve genotype-1 HCV$^+$ (CHC) patients were obtained. In HD, TRAF1 expression on CD8$^+$ cells according to CD45RO/CCR7 phenotype was tested after either αCD3/αCD28 or IL-7 stimulation. On HCV-specific CTLs from SVR and CHC, directly *ex-vivo* TRAF1/CD127 expression was tested and proliferation ability and TRAF1 level after specific stimulation without any treatment and in presence of IL-7 and/or 4-1BBL was checked. HCV-specific CTLs were visualised by staining with labelled HLA-A2/epitope multimeric complexes (Pentamer) and CD8 mAb. TRAF1/CD45RO/CCR7/CD127 labelling was performed by staining with different mAb. Flow-cytometric analysis was carried-out.

Results: On total CD8$^+$ cells from HD, TRAF1 expression was significantly higher on naïve and central memory T cells than in RA and RO effector memory T cells after αCD3/αCD28 *in-vitro* challenge (p < 0.05). Interestingly, IL-7 *in-vitro* treatment increased TRAF1 expression on all CD8 populations (p < 0.05). HCV-specific CTLs (CD8$^+$/Pentamer$^+$) from CHC showed lower CD127 and TRAF1 expression than in SVR (p < 0.01 and p < 0.05, respectively). A positive correlation between CD127 and TRAF1 on CD8$^+$/Pentamer$^+$ cells was observed (r = 0.7, p < 0.05). HCV-specific CTL proliferation after specific *in-vitro* challenge was lower in CHC than in SVR (p < 0.05). Specific *in-vitro* simulation in presence of IL-7/4-1BBL improved HCV-specific CTL proliferation ability in CHC, showing a similar post-treatment TRAF1 expression than SVR, while treatment with either IL-7 or 4-1BBL alone induced a lower effect (p < 0.05).

Conclusions: TRAF1 expression is down-regulated on exhausted T cells but IL-7 can induce its up-regulation. In CHC, there is a TRAF1/CD127 down-regulation associated to low reactivity on HCV-specific CTLs. IL-7/4-1BBL treatment can improve HCV-specific CTL response in CHC by TRAF1/4-1BB signalling enhancement.

Non-invasive markers of liver fibrosis

P0507
EFFECTS OF VITAMIN D SUPPLEMENTATION ON LIVER STIFFNESS IN PATIENTS WITH CHRONIC LIVER DISEASES

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Background and Aims: Vitamin D deficiency is common in patients with chronic liver diseases (CLD) and unfavorable genetic polymorphisms of genes controlling vitamin D metabolism have been associated with increased liver stiffness in patients with CLD (Grünhage et al. Hepatology 2012; 56: 1883–91). No data on the effect of vitamin D supplementation on liver stiffness is available. Our aim was to analyse patients with CLD with respect to vitamin D and liver phenotypes.
Methods: Patients were randomly retrieved in our hospital data management system and included if they fulfilled the characteristics of at least one of the following groups. Group 1 consists of patients with vitamin D deficiency (<20 ng/ml) who received vitamin D supplements; group 2 was defined as patients with deficiency who did not receive any supplementation, and group 3 comprised patients without vitamin D deficiency (vitamin D levels >20 ng/ml). Patient characteristics such as age, gender, etiology of liver disease as well as baseline parameters (liver stiffness, AST, ALT, GGT) and follow-up parameters were analysed in pairwise comparisons.
Results: We included 100 patients in groups 1 and 2 but we were only able to identify 33 patients with CLD who presented with normal vitamin D levels without supplementation, reflecting the increased incidence of vitamin D deficiency in this population. In all three groups, patients with chronic hepatitis C virus infection represented the largest subgroup (41%, 55%, 64%). The supplementation of vitamin D for at least 6 months resulted in a significant rise of vitamin D levels in group 1 (10.6±5.2 vs 32.9±14.8). Interestingly, these patients also presented with a significant decrease in median liver stiffness (16.0±19.9 kPa vs. 12.9±11.7 kPa; $P = 0.02$), while no significant changes were detected in pairwise comparisons in groups 2 and 3 (both $P > 0.05$). Interestingly, ALT declined significantly in patients with supplementation (66±74 U/L vs. 49±41 U/L, $P = 0.01$), but also in patients of group 2 (67±80 U/L vs. 49±49 U/L; $P = 0.03$) but patients without vitamin D deficiency and without supplementation showed no change ($P > 0.05$).
Conclusions: Our data indicate that vitamin D supplementation might have an influence on liver stiffness in patients with CLD independent from improvement of liver function tests, consistent with our findings in preclinical models (Hochrath et al. 2014). Prospective trials are mandatory to assess the therapeutic potential of vitamin D in patients with chronic liver diseases.

P0508
BIOPSY FINDINGS, ACE LEVELS AND APRI ARE SIMILAR IN PATIENTS WITH SARCOIDOSIS WITH AND WITHOUT PORTAL HYPERTENSION

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Background and Aims: *Background:* Sarcoidosis of the liver is a cause of portal hypertension both in the in the presence of cirrhosis as well as its absence (pre-sinusoidal portal hypertension). There are no prospective studies evaluating portal hypertension in hepatic sarcoidosis and its correlation with histology and non-invasive markers of fibrosis.
Methods: We prospectively evaluated 21 consecutive patients with hepatic sarcoidosis seen at the Cleveland clinic referral center for sarcoidosis. All patients were evaluated for portal hypertension by imaging as well as percutaneous or trans-jugular liver biopsies. Biopsies were scored blindly by a single liver pathologist. Various non-invasive markers of portal hypertension were evaluated.
Results: Twenty-nine percent (n = 6) of subjects had evidence of portal hypertension (2 had intrahepatic collaterals and 4 had pressure gradient >5 mmHg), four of whom had non-cirrhotic portal hypertension. There was no difference in liver enzymes, Angiotensin Converting Enzyme (ACE) levels or AST to Platelet ratio (APRI) between patients with and without portal hypertension.
Conclusions: In a prospective study of patients with hepatic sarcoidosis, 21% had evidence of portal hypertension and 14% had non-cirrhotic portal hypertension. There was no difference in liver enzyme abnormalities, ACE levels or APRI between patients with and without portal hypertension.

P0509
DIAGNOSTIC AND PROGNOSTIC STAGINGS ARE IMPROVED BY MULTI-TARGETING BIOMARKERS. APPLICATION TO LIVER FIBROSIS TESTS

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Background and Aims: Construction of diagnostic tests is usually limited by a unique diagnostic target, e.g. significant liver fibrosis in chronic liver diseases. Our aim was to improve diagnostic accuracy of diagnostic tests by simultaneously targeting biomarkers for different diagnostic targets in the context of liver fibrosis tests.
Methods: A total of 3247 patients were included: 1688 patients with chronic hepatitis C in two diagnostic populations with Metavir fibrosis stages (F) on liver biopsy as reference, and 1559 patients with miscellaneous chronic liver diseases with prognostic outcome (liver related death) as reference. We tested 12 biomarkers provided by different published single-targeted blood tests. Construction of new multi-targeted test included the four following steps: classical construction of four single-targeted tests corresponding to four Metavir targets; three single-targeted tests were independent predictors of fibrosis level; fibrosis classifications were then derived from the three test scores; test classifications were segmented and the three most accurate parts were aggregated in a new unique classification of a multi-targeted test called MFM.
Results: In the derivation diagnostic population, the accuracy of multi-targeted MFM test classification was (predominant/minority F stage): F0/1: 96.0%, F1/2: 85.7%, F1/2/3: 95.7%, F2/3/4: 86.6%, F3/4: 86.3%, F4: 96.0%. The overall accuracy of MFM test classification was 92.7% vs. 87.6% for reference published single-targeted test ($p < 0.001$). MFM sensitivity for cirrhosis of the 3 classes including F4 was 91.3% and MFM positive predictive value for cirrhosis of F4 class was 96.0%. In the validation diagnostic population, overall diagnostic accuracy of MFM test was 88.2% vs. 83.6% for published single-targeted test ($p < 0.001$). In the prognostic population, multi-targeted MFM classification well predicted liver related death (log rank test for Kaplan–Meier plots: $p < 10^{-5}$), especially better between early (F3/4) and definitive cirrhosis (F4, $p = 0.0003$) than with published single-targeted test ($p = 0.0034$).
Conclusions: The classical way to construct a diagnostic test can be significantly improved by multi-targeting biomarkers. Applied to

fibrosis tests, this improves accuracy – up to 93% – and prognostic discrimination especially between early and definitive cirrhosis.

P0510

DIRECT COMPARISONS OF FIBROTEST, APRI, FIB-4, AND TRANSIENT ELASTOGRAPHY (TE) FOR THE DIAGNOSIS OF CIRRHOSIS AND FIBROSIS, IN PATIENTS WITH CHRONIC HEPATITIS C (CHC) AND B (CHB) USING INTENTION TO DIAGNOSE AND BAYESIAN METHODS. A SYSTEMATIC REVIEW

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Background and Aims: The diagnostic performances of non-invasive tests for the diagnosis of cirrhosis (F4) or clinically significant fibrosis (METAVIR stages F2F3F4) in chronic liver disease (CLD) have been assessed using indirect comparisons and "per-protocol" (PP) analysis, that is, without taking into account the applicability (failure or non reliability) of tests, ["intention-to-diagnose" (ITD)]. As for drugs, indirect comparisons between tests are limited by the spectrum effect and PP analysis by applicability effect. The aim was to compare the performance of the four most validated tests (FibroTest, TE, APRI and FIB-4) in patients with chronic liver diseases using only direct comparison, ITD and Bayesian methods.
Methods: Direct comparisons using biopsy as reference were searched (MEDLINE) from 2001 to 2014; four methods were used for AUROCs' difference (AUC-D) in ITD: the descriptive AUC-D (Chou 2013), pooled indirect AUC-D, pooled direct AUC-D and pooled direct Bayesian AUC-D (BayesAUC-D).
Results: Among 1279 biomarker studies identified, 71 studies with 77 groups of patients were included (37 with CHC only, 28 CHB only, and 12 "MixedCB" defined as CHC-CHB >49% of CLD) allowing 185 direct comparisons between the 4 tests' AUROCs; 99 for F2F3F4 (12,725 patients) and 86 for F4 (10,929 patients). The significant (credibility interval) BayesAUC-D were 0.06 in favor of FibroTest vs TE and 0.05 vs APRI; for cirrhosis BayesAUC-D were 0.07 in favor of TE vs APRI and 0.04 for FIB4 vs APRI. Non-applicability rate (median/range) was 0% (0–8%) for FibroTest lower ($P < 0.0001$) than for TE 8% (0–41.8); APRI and FIB4 have no rules for applicability.

Table: Fibrosis biomarkers' performances in CHC and CHB, assessed by direct comparisons, intention to diagnose and Bayesian methods

Stage of fibrosis	Test A	Test B	Studies n	Median AUROC A/B	Descriptive ADC Median difference (range)	Pooled Bayesian AUROC difference [$CI_{95\%}$]	Bayesian Credible Conclusion
F2F3F4	FibroTest	TE	15	0.79/0.77	0.04 (−0.10; 0.14)	0.06 [0.02; 0.09]	FibroTest > TE
	FibroTest	APRI	21	0.80/0.73	0.05 (−0.06; 0.18)	0.05 [0.03; 0.07]	FibroTest > APRI
	FibroTest	FIB-4	5	0.79/0.74	0.03 (−0.01; 0.08)	0.02 [−0.05; 0.10]	
	TE	APRI	17	0.82/0.78	0.01 (−0.14; 0.25)	0.04 [0.00; 0.09]	
	TE	FIB-4	6	0.81/0.74	0.04 (−0.13; 0.15)	0.03 [−0.08; 0.15]	
	APRI	FIB-4	35	0.74/0.75	−0.01 (−0.13; 0.10)	−0.01 [−0.03; 0.01]	
F4	FibroTest	TE	13	0.85/0.83	0.01 (−0.18; 0.07)	0.00 [−0.04; 0.04]	
	FibroTest	APRI	14	0.83/0.78	0.08 (−0.13; 0.35)	0.05 [0.00; 0.11]	
	FibroTest	FIB-4	6	0.83/0.81	0.05 (−0.03; 0.11)	0.05 [−0.03; 0.12]	
	TE	APRI	18	0.87/0.81	0.08 (−0.07; 0.37)	0.07 [0.02; 0.13]	TE > APRI
	TE	FIB-4	7	0.87/0.77	0.13 (−0.01; 0.25)	0.12 [0.00; 0.24]	
	APRI	FIB-4	28	0.78/0.82	−0.04 (−0.24; 0.10)	−0.04 [−0.05; −0.02]	FIB4 > APRI

Conclusions: This overview, the first focusing on direct comparisons in ITD and using Bayesian meta-analysis, permitted to compare fibrosis biomarkers without the limitations of meta-analyses not taking into account the tests' applicability, and of indirect comparisons. FibroTest had better performance than TE for the diagnosis of F2F3F4, in CHC and CHB, which was not observed by indirect and per-protocol previous meta-analyses.

P0511

DIRECT COMPARISON OF 3 ELASTROMETRY DEVICES (FIBROSCAN, ACOUSTIC RADIATION FORCE IMPULSE, SUPERSONIC SHEARWAVE IMAGING) FOR THE NON-INVASIVE DIAGNOSIS OF LIVER FIBROSIS IN CHRONIC LIVER DISEASES

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Background and Aims: Liver stiffness measurement using elastography allows for a non-invasive diagnosis of liver fibrosis with immediate results at bedside. The first elastography device developed was Fibroscan (FS) using transient elastography with mechanical impulse on the skin. Then, acoustic-based elastographic methods, Acoustic Radiation Force Impulse (ARFI) and more recently Supersonic Shearwave Imaging (SSI), have been developed using ultrasound pulses. We aimed to evaluate and compare the feasibility and the diagnostic accuracy of FS, ARFI, and SSI for the non-invasive diagnosis of liver fibrosis.
Methods: 192 patients with chronic liver disease, liver biopsy, FS, ARFI and SSI were included. Metavir F staging on biopsy was taken as the reference for liver fibrosis. Result for each elastographic device was the median of 10 valid measurements. Diagnostic cut-offs were calculated to maximize the sum of sensitivity + specificity.
Results: Cause of chronic liver disease was NAFLD in 55.7% of cases, viral hepatitis: 16.1%, alcohol: 16.7%, and others: 11.5%. Fibrosis stage prevalence was: F0: 23.4%, F1: 37.0%, F2: 19.3%, F3: 13.5%, F4: 6.8%. Failure of liver stiffness measurement (no valid measurement) occurred in 18 patients (9.4%) with FS, no patients with ARFI, and 3 patients (1.6%) with SSI ($p = 0.001$ between FS and SSI). Results for the 3 devices were available in 171 patients. Obuchowski indexes were: FS: 0.855±0.018, ARFI: 0.761±0.027, SSI: 0.789±0.025 (FS vs ARFI or SSI: $p \leq 0.020$). AUROC for significant fibrosis (Metavir F≥2) were: FS: 0.863±0.027, ARFI: 0.749±0.039, SSI: 0.781±0.036 ($p = 0.006$; FS vs ARFI or SSI: $p \leq 0.021$). Diagnostic cut-offs for F≥2 were: FS: 8.0 kPa, ARFI: 1.29 m/s, SSI: 1.85 m/s. Using this cut-offs, diagnostic accuracy for F≥2 was: FS: 76.0%, ARFI: 70.2%, SSI: 77.2% ($p = 0.204$). AUROC for cirrhosis were: FS: 0.941±0.027, ARFI: 0.895±0.048, SSI: 0.870±0.035 ($p = 0.011$; FS vs SSI: $p = 0.010$). Diagnostic cut-offs for cirrhosis were: FS: 16.6 kPa, ARFI: 1.87 m/s, SSI: 1.93 m/s. Using this cut-offs, diagnostic accuracy for cirrhosis was: FS: 90.6%, ARFI: 79.5%, SSI: 75.4% ($p < 0.001$, FS vs others: $p \leq 0.001$).
Conclusions: ARFI and SSI have better feasibility and similar accuracy for the diagnosis of significant fibrosis than FS. However, FS has the best accuracy for the diagnosis of cirrhosis.

P0512

LIVER STIFFNESS MEASURMENTS USING FIBROSCAN AFTER THREE MONTHS OF IFN-BASED ANTIVIRAL THERAPY IS UNLIKELY TO PREDICT VIRAL RESPONSE IN CIRRHOTIC PATIENTS

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Background and Aims: Chronic HCV infection (CHC) is the leading cause of mortality from liver cirrhosis and hepatocellular carcinoma. Antiviral therapy can prevent disease progression. Transient Elastografy (TE; Fibroscan) is an accurate surrogate marker to liver fibrosis, by measuring liver stiffness (LS). LS decrease has been associated with sustained virologic response (SVR). In this study we aimed to assess the changes of LS measurments in CHC patients during and one year after Interferon (IFN)-based antiviral therapy (IFN/ribavirin) or (telaprevir+IFN/ribavirin).
Methods: This is an ongoing study, in which consecutive 69 CHC patients (53.6% females, mean age 57.9±11.4) who underwent

antiviral therapy for at least 20 weeks were enrolled. LS was measured using Fibroscan at baseline, after three months, at the end of treatment and one year after treatment discontinuation. Fibrosis was graded using METAVIR score.
Results: Twenty patients were treated with triple therapy and 49 with IFN/ribavirin. Fifty patients had SVR and 19 were non-responders. LS changed significantly in all patients and independently from the initial fibrosis stage, genotype, type of treatment, basal ALT and BMI. Twelve months after treatment discontinuation, we found that in SVR patients: F0–F1, F2 and F3 patients (39.1%, 7.2% and 17.4%; respectively) showed no significant LS decrease (P = 0.186, 0.068 and 0.075; respectively). Conversely, in F4 patients (36.2%) LS was significantly decreased (P = 0.015) after one year of treatment completion. In all patients with no SVR, no significant decrease in LS was observed. Interestingly, all Patients with F4 fibrosis (even non-responders) showed an initial significant decrease in LS (P = 0.024) at 3 months after the start of treatment. However, this decrease was not predictive of SVR; area under the ROC curve 0.369 (CI %: 0.145–0.592) P = 0.265.
Conclusions: Performance of LS measurements for fibrosis assessment confirmed results from previous studies. Our preliminary data suggest that LS changes significantly in CHC patients treated with IFN-based antiviral therapy (standard and triple therapy with telaprevir) and it decreases significantly in responders with high initial LS measurements independently from the type treatment. Initial significant decrease in LS measurements in such patients is unlikely to predict an SVR.

P0513
DIAGNOSTIC ALGORITHM FOR IMPLEMENTATION OF NON-INVASIVE SCORES FOR LIVER FIBROSIS IN CLINICAL PRACTICE IN CHILDREN WITH CHRONIC HEPATITIS C
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Background and Aims: Considering that most noninvasive scores for evaluating liver fibrosis have been validated only in adults and taking into account that liver biopsy is less accepted in children. there is a clear need to assess these scores in childrenn. the aim of our study was to assess the diagnostic performance of 3 of the non-invasive fibrosis scores with limited expense and simple calculation and then combined them with the aim of defining an algorithm of higher diagnostic accuracy (DA) in Egyptian children with chronic hepatitis C (CHC).
Methods: 117 children with CHC aged 5–15 years were evaluated for Forns score, FIB-4 and APRI at the time of liver biopsy and a combination algorithm for discriminating non-significant (F0–F1) versus significant (F2, F3F4) fibrosis were developed. Liver fibrosis staging were assessed according to the METAVIR scoring system. The performance of each scores is expressed by the area under the receiving-operating-characteristic (AUROC). Positive and negative predictive values (PPV, NPV) were calculated using cut-off generated for each score according to the highest Youden index to provide the best DA.
Results: Fibrosis stages were: F0: 47%, F1: 39% and F2: 14%. The studied scores had a similar diagnostic performance (AUROCs 0.79–0.76). All scores showed excellent NPV (>93%) to exclude significant fibrosis, while PPV was quite low for all scores (30–40%), so that significant fibrosis cannot be reliably predicted in HCV studied children. Several combinations were tested and the best algorithm was to first calculate APRI in all children followed by Forns score in children classified F2 by APRI. The algorithm we have developed was based on the NPV of the scores, identifying children with non-significant fibrosis. In this algorithm APRI (cut-off <0.68) correctly classified 86% of children with non-significant fibrosis, in the second step Forns score (cut-off <0.97) correctly classified another 6% of children with non-significant fibrosis and restricting liver biopsy to children classified F2 by both scores.
Conclusions: Studied non-invasive scores, when combined, may reduce by 85% the number of liver biopsies needed for correctly classifying hepatic fibrosis in children. Being non-invasive, they can be repeated every year to monitor liver fibrosis progression in those children with non-significant fibrosis. However, liver biopsy cannot be completely avoided but should be considered when significant fibrosis is predicted by the non-invasive scores.

P0514
THE CORRELATION BETWEEN 25-HYDROXYVITAMIN D LEVEL AND LIVER FIBROSIS ASSESSED BY TRANSIENT ELASTOGRAPHY IN PATIENTS WITH COMPENSATED CHRONIC LIVER DISEASE
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Background and Aims: 25-hydroxyvitamin D [25(OH)D_3] deficiency has been known to be prevalent in chronic liver disease (CLD). Low 25(OH)D_3 serum levels were correlated with an increase of liver fibrosis. The purpose of this study was to evaluate correlation between 25(OH)D_3 level and liver fibrosis assessed by transient elastography (TE) in patient with compensated CLD.
Methods: From January 2013 to June 2014, the measurement of 25(OH)D_3 serum levels and liver stiffness using TE were done in 260 CLD patients. Among them, 207 CLD patients were enrolled in this study after excluding the patients with decompensated liver disease.
Results: The mean age of patients was 48 years and 151 (72.9%) patients were male. The most common etiology was chronic hepatitis B (110, 53.1%), followed by chronic hepatitis C (15, 7.2%), non-alcoholic fatty liver disease (25, 12.1%), alcohol (41, 19.8%) and others (16, 7.7%). The median liver stiffness value was 6.8 kPa (Inter-quartile range: 5–11.5) and mean 25(OH)D_3 level was 12.68 ng/ml (standard deviation: 9.1–18.57). The number of patients with 25(OH)D_3 deficiency (<20 ng/ml), severe 25(OH)D_3 deficiency (<10 ng/ml) were 94 (45.4%) and 72 (34.8%) respectively. Advanced liver fibrosis defined by TE (9.5 kPa) was 75 (36.2%). There was a significantly correlation between 25(OH)D_3 deficiency and liver stiffness ($r = -0.204$, $p < 0.003$). On the multivariate analysis, associated with advanced liver fibrosis severe 25(OH)D_3 deficiency [adjusted odds ratio (aOR) 3.92, 95% confidence interval (CI) 1.70–9.01, $p = 0.001$], Gamma GT [aOR 1.004, 95% CI 1.00–1.007, $p = 0.041$], and FIB4 [aOR 2.01, 95% CI 1.43.-2.83, $p < 0.001$] were independently significant factors.
Conclusions: In patients with compensated CLD, there was a close correlation between 25(OH)D_3 level and liver stiffness assessed by TE. Severe vitamin D deficiency was independently associated with advanced liver fibrosis.

P0515

BIOLOGICAL STABILITY AND DIAGNOSTIC ACCURACY OF ELF® (ENHANCED LIVER FIBROSIS TEST) TO IDENTIFY LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C USING CRYOPRESERVED SERUM SAMPLES DURING 25 YEARS

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Background and Aims: Applicability of liver fibrosis serum markers is higher than other non-invasive methods. Moreover, cryopreservation of serum samples is a routine procedure in reference centers.
To evaluate the biological stability of serum markers and their diagnostic accuracy to identify significant fibrosis using HCV infected frozen serum samples during the last 25 years.
Methods: We evaluated 225 frozen serums samples of patients with chronic HCV infection and their liver biopsy during 5 periods: 1990–94 (n = 25), 1995–99 (n = 50), 2000–04 (n = 50), 2005–09 (n = 50) and 2010–14 (n = 50). Thirty-four patients with low quality biopsies (<6 portal tracts) or more tan 2 years between biopsy and serum sample were excluded. Hyaluronic acid (HA), N-terminal propeptide of type III procollagen (PIIINP) and tissue inhibitor of metalloproteinases 1 (TIMP-1) were measured to determine ELF® (Enhanced Liver Fibrosis) using ADVIA Centaur® immunochemical analyzer. We evaluated the diagnostic accuracy (AUROC) of ELF® to identify significant fibrosis (F2–4).
Results: Seventy-six patients (39.8%) had mild fibrosis (F0–1), 37 (22%) moderate (F2) and 82 (38.2%) advanced liver fibrosis (F3–4). Length of biopsy (median) for 1990–94 (14 mm) was lower than the rest of periods (18 mm) ($p < 0.001$). Values of HA, PIIINP and TIMP-1 were similar during all periods (1990–2014) without a decline of their levels. We did not found differences of ELF® values (median) between periods (1995–2014) in patients with mild fibrosis (8.4–8.7), moderate (9.8–10) or advanced fibrosis (10.4–11.2) (p = ns between periods and fibrosis stages). The AUROCs of ELF® to identify significant fibrosis were from 0.86 to 0.91 for the different periods (1995–2014).
Conclusions: Biological stability of direct serum markers (HA, PIIINP and TIMP-1) and diagnostic accuracy of ELF® to identify significant fibrosis was very high using HCV infected frozen serum samples during the last 20 years.

P0516

QUALITY CONTROL OF QUANTIFICATION OF ARFI MEASUREMENTS – PHANTOM STUDY IN COMPARISON WITH FIBROSCAN

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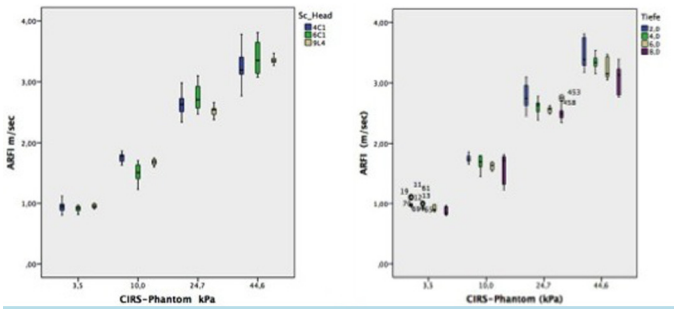
Background and Aims: A precise and reproducible non-invasive assessment of the progression of liver fibrosis is important for the estimation of prognosis and treatment decisions. Alternative approaches include transient elastography (FibroScan®) and shear wave measurements (ARFI technique). Although there are reports demonstrating high correlations with histology and between different techniques, absolute values and reproducibility are still under discussion. The aim of our study was to evaluate the reproducibility and factors influencing the absolute values using a standardized phantom.
Methods: For validation, all examinations were correlated with the CIRS shear wave phantom (039) made of Zerdine with values for Young's modulus between 3 and 48 kPa (attenuation 0.5 dB/cm/MHz, speed 1560–1600 m/s, density 1.03 g/cc). Homogeneity of the phantom was proved by CT and 2D shear wave imaging. ARFI measurements were performed with S2000 and S3000 machines (Siemens) using different scan heads (9 MHz, 4 and 6 MHz), depths, pressures and angles in at least 10 repeated measurements. The ARFI values (m/s) were correlated with transient elastography (kPa) and the expected SVI values of the CIRS phantom. Because the first series showed an underestimation of all modalities, the phantom was redesigned by CIRS with values of 3.5, 10.0, 24.7 and 44.8 kPa.
Results: Although the correlation in the first series was 0.89, there was an underestimation of shear wave velocities and stiffness values with all ultrasound machines by 15–40%. Using the redesigned phantom, the correlation improved to 0.96. The underestimation of transient elastography ranged from 14 to 31%; the underestimation of ARFI values was in the range of 0.3 to 10.2% with lowest errors when using the 4 MHz scan head. Critical factors are examination pressure (ARFI) and depth of measurement (Figure 1).
Conclusions: Although there was a significant correlation between phantom values and ARFI measurements, there was a systematic underestimation of the absolute values. The best results for measurements in liver-equivalent depths were achieved at 4 MHz. Depth causes a significant reduction of the shear wave velocity measurements with low errors in the range of 3–6 cm depth. Our experimental results provide evidence that there is still a problem in defining the gold standard for non-invasive liver stiffness measurements.



Phantomangaben		Fibroscan		4C1		6C1		9L4	
kPa	m/sec	kPa	Abw. %	m/sec	Ab. %	m/sec	Abw.%	m/sec	Abw. %
3,5	1,08	2,4	-31,43	1,03	-4,64	0,97	-10,2	0,98	-9,27
10	1,83	8,6	-14	1,82	-0,31	1,7	-6,89	1,7	-6,89
24,7	2,87	21,1	-14,57	2,85	-0,68	2,77	-3,46	2,58	-10,09
44,6	3,86	60,2	34,98	3,46	-10,26	3,79	-1,7	3,35	-13,12

Figure 1.

P0517

FEASIBILITY OF TRANSIENT ELASTOGRAPHY IN OVERWEIGHT AND OBESE PATIENTS

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Background and Aims: Liver stiffness measurement (LSM) using transient elastography (TE) is a non-invasive technique that evaluates liver fibrosis. However reliable elasticity measurements are difficult to obtain in overweight (BMI≥25 kg/m^2) and obese (BMI≥30 kg/m^2) patients with the M probe. The XL probe was meant to improve the feasibility of TE in this category of patients. *The aim* of our paper was to assess the feasibility of the TE in daily practice in overweight and obese patients.
Methods: Our study included 2046 successive overweight and obese patients with chronic hepatitis and cirrhosis of various

etiologies in which TE measurements were made either with the M (3.5 MHz) probe or with XL (2.5 MHz) between January 2012 and October 2014. In patients in which reliable measurements could not be obtained by M probe, XL measurements were performed in the same session. In each patient 10 valid LSM were acquired with each probe, a median was calculated expressed in kiloPascals (kPa). Reliable measurements were defined as: median value of 10 valid LSM with a success rate (SR) ≥60% and an interquartile range interval (IQR) <30%. Unreliable TE measurement were considered the following situations: fewer than 10 valid shots; SR <60% and/or IQR ≥30%.

Results: A total of 2046 patients were examined, 1156 (56.5%) overweight and 890 (43.5%) obese. In the 1156 overweight patients reliable LSM were obtained in 979 (84.7%) cases: in 729 (63.1%) by the standard M probe and in 250 (21.6%) by the XL probe. In the 890 obese patients, reliable measurements were obtained in 744 patients (83.6%), in 165 (18.5%) by the M probe, and in 522 (58.6%) by using the XL probe. Thus, by using both probes, reliable measurements were obtained in 1723 (84.2%) of the 2046 examined cases.

Conclusions: By using both M and XL probes, reliable LSM by TE can be obtained in the majority of the obese and overweight patients (84.2%).

P0518

ARTERIAL PRESSURE SUFFICES TO INCREASE LIVER STIFFNESS INDEPENDENT OF CENTRAL VENOUS PRESSURE

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Background and Aims: Liver stiffness (LS) is not only increased by matrix deposition (fibrosis stage) but also in response to an elevated central venous pressure (liver congestion) and many other more complex conditions such as inflammation or food intake. We here study whether arterial or portal pressure is able to modulate LS.

Methods: LS was measured in 25 male Wistar rats using transient elastography (Microfibroscan, Echosens, Paris) during single i.v. injections of Epinephrine (0.05 mg), Norepinephrine (4 μg), Dobutamine (50–150 mg) and sodium chloride (1–4 ml, control). Pressures in all liver-relevant compartments (abdominal aorta, caval and portal vein) were invasively measured in real time using the Powerlab device and analyzed with Labchart software (AD Instruments, New Zealand).

Results: Initial normal LS values in rats were comparable to those in humans with a mean of 4.1 kPa. Epinephrine and Norepinephrine drastically increased systolic arterial pressures from 120 to >210 mmHg while LS doubled from 4 to 8 kPa. In the same time, central venous pressure (CVP) remained unchanged while portal pressure slightly increased by a mean of 3 mmHg. In confirmation, LS correlated highly with arterial pressures ($r = 0.811$) and portal pressure ($r = 0.599$) but not with CVP. Real-time monitoring clearly demonstrated that the slight elevation of portal pressure followed the increase of LS with a lack phase of 8 seconds. Both Dobutamine and volume challenge with sodium chloride of up to 4 ml failed to increase LS.

Conclusions: We here show that an increase of the arterial pressure is sufficient to elevate LS independent of the central venous pressure and prior to the portal pressure. Thus, elevated arterial pressure could be an important cofactor for the observed increase of LS e.g. during inflammation or food intake.

P0519

LIPIDOMICS REVEALS THAT LOW PLASMA UNSATURATED TO SATURATED FAT RATIOS ARE BIOMARKERS OF NAFLD AND LIVER DAMAGE

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Background and Aims: Alterations in lipid metabolism and insulin resistance (IR), have been associated with development and progression of NAFLD. Increased de novo lipogenesis (DNL) and low activity of Stearoyl-CoA desaturase-1 (SCD1) result not only in fatty liver but also in unbalanced production of unsaturated vs. saturated fat, ultimately determining lipotoxicity. Other classes of fat, e.g., ceramides, have been associated with organ inflammation and lipotoxicity. Thus, we measured plasma lipidomic profile and evaluated the association with NAFLD and liver fibrosis.

Methods: We studied 54 subjects, 45 non obese NAFLD with liver biopsy and 9 controls (CT) without fatty liver and measured different plasma lipid classes (i.e., FFA; lysophosphocholine, Lyso-PC; Ceramides, Cer) by mass spectrometry (GC-MS and LC-MS QTOF). We evaluated unsaturated to saturated fat ratio in FFA (PUFA/SFA) and Lyso-PC, hepatic TG (by MRI), indexes of adipose tissue IR (AT-IR= fasting lipolysis x insulin); hepatic IR (Hep-IR=EGPxInsulin); de novo lipogenesis index (DNL=16:0/18:2); SCD1 activity (16:1/16:0); MCP-1; oxidized LDL (ox-LDL), adiponectin, tryglicerides and GSG-index [= glutamate/(glycine + serine)] that we recently developed and found associated with presence of oxidative stress and glutathione demand, but also with TCA cycle turnover

Results: PUFA/SFA (in both FFA and Lyso-PC) were decreased in NAFLD vs CT particularly in NAFLD with fibrosis score F3–F4 ($p < 0.05$ vs F0 and CT). Fatty acid PUFA/SFA were inversely associated with hepatic TG ($r = -0.56$), AT-IR ($r = -0.37$), Hep-IR ($r = -0.28$), DNL ($r = -0.93$), SCD1 ($r = -0.38$), GSG ($r = -0.52$), and MCP-1 ($r = -0.42$, all $p < 0.05$). Lyso-PC PUFA/SFA were inversely associated with hepatic TG ($r = -0.44$), GSG ($r = -0.42$) and ox-LDL ($r = -0.30$). Total ceramides were increased in NAFLD vs CT, and Cer(d18.0/18:1) and Cer(d18:0/24:0) were also increased in F3–F4 vs F0 and CT ($p < 0.05$). Cer(d18:0/24:0) and Cer(d18.0/18:1) were positively associated with hepatic TG ($r = 0.43$, $r = 0.30$), DNL ($r = 0.34$, $r = 0.39$), plasma TG ($r = 0.42$, $r = 0.41$), AT-IR ($r = 0.46$, $r = 0.31$) and oxLDL ($r = 0.51$, $r = 0.40$).

Conclusions: Decreased PUFA/SFA ratio and increased ceramides are markers of metabolic derangement (i.e., increased lipotoxicity, hepatic fat accumulation and de novo lipogenesis) and severe liver fibrosis in NAFLD.

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P0520

ACCEPTABILITY, RELIABILITY AND APPLICABILITY OF LIVER BIOPSY AND NON-INVASIVE METHODS FOR ASSESSMENT OF HEPATIC FIBROSIS AND CIRRHOSIS AMONG HEPATOLOGISTS; A WEB BASED SURVEY

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Background and Aims: Liver biopsy is the standard reference for staging hepatic fibrosis. Non-invasive methods for assessment of hepatic fibrosis and cirrhosis are becoming increasingly popular. We aimed at exploring the change in practice regarding the use of liver biopsy and non-invasive methods for staging hepatic fibrosis and cirrhosis.
Methods: A 56 questions survey was designed and hosted on an online survey's hosting website. Invitations sent to practicing physicians interested in hepatology via e-mails and through major international hepatology societies' social media walls (Twitter, Facebook and LinkedIn). Questions covered different aspects of practice regarding the use of liver biopsy, serum fibrosis biomarkers and radiological methods for assessment of hepatic fibrosis and cirrhosis.
Results: 444 physicians from 28 countries completed the survey (70% hepatologists and gastroenterologists, 69% experts or consultants). Liver biopsy is still considered the gold standard for assessment of hepatic fibrosis and cirrhosis by 85% of participants. Liver biopsy was accepted by 76.33% of their patients, 97.6% of participants thought that liver biopsy result was reliable and 60.84% admitted that liver biopsy was applicable for all patients. 94.23% reported the need of a more practical alternative to liver biopsy to assess disease progression or response to treatment. 91.89% of participants knew serum biomarkers, 93.36% reported they were acceptable by patients, 85.26% thought they were reliable and 57.9% applied them. 95.4% were familiar with radiological methods of non-invasive assessment of hepatic fibrosis, 97.81% reported that radiological methods were acceptable by their patients, 94.96% thought that they were reliable and 96.28% reported they were applicable in clinical practice. 86.72% thought that combining non-invasive methods was better than using a single method and 39.41% of them thought that radiological methods were cheaper than biomarkers (35.59%), combination of both (24.55%) or liver biopsy (0.45%). 96.17% of them agreed that we are in need of a guideline for using non-invasive methods for assessment of liver fibrosis.
Conclusions: Acceptability of non-invasive methods for assessment of hepatic fibrosis by patients was better than liver biopsy. Physicians trusted liver biopsy more than radiological methods or serum biomarkers and applied non-invasive radiological methods easily to their patients than liver biopsy or serum biomarkers.

P0521

CHARACTERIZATION AND VALIDATION OF ANGIOGENIC GENETIC VARIANTS RELATED TO CHRONIC HEPATITIS C PROGRESSION TO HEPATOCELLULAR CARCINOMA

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Background and Aims: Chronic hepatitis C (CHC) progression deeply influences hepatic vascular homeostasis leading to significant functional and anatomical alterations of the liver. As a consequence of CHC advancement, patients show diverse grades of inflammation, fibrosis and angiogenesis which confer an important risk of cirrhosis and hepatocellular carcinoma (HCC) development. Evolution of CHC is highly variable among patients depending on a broad set of host factors, highlighting the importance of patient's genetic profile. Therefore, the assessment of the overall contribution of certain genetic variants related to main vascular mediators on CHC progression might be crucial for treatment decision making and personalized management of CHC.
Aims: To test and validate the impact of angiogenic-related genetic variants on CHC progression to cirrhosis and HCC.
Methods: 364 SNPs were selected by their potential effects on gene expression or functionality from 76 candidate genes (angiogenic factors, receptors and signalling effectors) and subsequently genotyped in 384 patients with fibrosis (F0–1=112, F2=43 and F3=49), cirrhosis (F4=45) and HCC (134) of CHC-etiology. Genotyping also included 20 additional SNPs previously related to CHC progression ($p < 0.01$) in an independent cohort ($n = 384$, 49th annual meeting of EASL, 2014) for their validation. Association of disease stages (F0–1 vs. F2–4, F0–1 vs. CHC and F2–4 vs. CHC) with genetic variants was analyzed by PLINK software and Bonferroni multiple test correction was applied.
Results: 13 SNPs located in 8 angiogenic genes were significantly associated with CHC progression ($p < 0.01$). Of those, 6 SNPs were related to significant fibrosis (F0–1 vs. F2–4), 2 to HCC progression (F2–4 vs. HCC) and 5 to the evolution from F0–1 to HCC. Interestingly, 2 of those SNPs associated with mild to significant fibrosis remained significantly associated even after Bonferroni correction ($pcB < 13 \times 10^{-5}$). Remarkably, 4 of the 20 SNPs previously associated with CHC progression by our group were independently validated in present study. Interestingly, one of those SNPs was related to significant fibrosis (F0–1 vs. F2–4) and HCC (F2–4 vs. CHC), 2 SNPs were associated with significant fibrosis (F0–1 vs. F2–4) and one SNP to HCC (F2–4 vs. HCC).
Conclusions: The influence of characterized and validated genetic variants on CHC progression to HCC might serve as a valuable tool for the clinical management of CHC patients and the early diagnostic of HCC.

P0522

VALID LIVER STIFFNESS MEASUREMENT CAN BE OBTAINED IN MORE THAN 99% OF PATIENTS WITH VIRAL HEPATITIS BY REPEATING THE EXAMINATION IN CASE OF INITIAL FAILURE

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Background and Aims: Liver Stiffness Measurement (LSM) with Fibroscan (Echosens) has become a standard tool for detecting liver fibrosis in patients with chronic viral hepatitis (CVH). Initially invalid LSM was reported in 10–20%. Since the introduction of a special XL-probe for obese patients and the new quality criteria for a valid LSM, the proportion of invalid LSM has decreased. The aim of this study was to investigate the value of performing additional LSM in patients with invalid LSM.
Methods: We evaluated the outcome of initial and repeated LSM at subsequent visits in two populations of patients with CVH, one with initial LSM before and one with initial LSM after the introduction of the XL probe. We determined the number of invalid scans according to the 2014 criteria for poorly reliable LSM: <10 valid measures or, IQR/Median >30% if LSM >7.1 kPa (Boursier J et al. Hepatology 2013).
Results: In the 3 years prior to the XL-probe we performed 1757 LSM among 746 patients with CVH. Overall 89.1% (1566/1757) of the LSM were valid using the new criteria and 91.3% (681/746) of the patients had a valid first LSM. At the second LSM 51.1% (23/45) of those with an initial invalid LSM had a valid examination. At subsequent examinations among patients with two invalid LSM, only 20% had a valid LSM. In the 4 years after introduction of the XL-probe in 2010, we performed 2701 scans among 970 patients (19.3% with the XL-probe). Overall 96.6% (2610/2701) of all the LSM were valid by new criteria and 96.3% of patients had a valid first LSM. Among 36 patients with an initial invalid LSM, 78.1% (25/32) had a valid scan at the second examination (4 were lost to follow-up). Of the 7 patients with two successive invalid scans, all examined (5) had a valid LSM at a later visit and the remaining 2 are awaiting examination. After the introduction of the XL-probe a valid LSM was obtained in 100% (21/21) who previously had only invalid examinations.
Conclusions: The success rate of LSM among patients with CVH is close to 100% when combining the use of the M- and/or XL-probe with repeated examination in patients with initial invalid LSM.

P0523

MODIFICATION OF EXPRESSION OF MIR-20A, MIR-92A AND MIR-122 DURING FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS C

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Background and Aims: The high stability of Micro-RNAs (MiRNAs) make them interesting as circulating biomarkers. They are released in the blood stream via cellular apoptosis and secretion. Patients with chronic hepatitis C (CHC) have a liver necrosis that can be detected by measuring transaminases. Thus, the relevance of studying microRNAs as biomarkers could be challenged in patients with CHC. In this study, we aimed to evaluate the potential of miRNAs as biomarkers of liver fibrosis in patients with CHC.
Methods: Serum samples and liver biopsies were available for respectively 86 and 40 patients. HCV-G1 were the more represented (58%). Among patients with available serum samples, the mean viral load was 1300×10^3 UI/mL. Fibrosis distribution was F1 (31.4%), F2 (20.9%), F3 (23.3%) and F4 (24.4%). Among patients with available biopsies, the mean viral load was 820×10^3 UI/mL and fibrosis distribution was F1 (27.5%), F2 (17.5%), F3 (27.5%) and F4 (27.5%). Fibrosis was staged according to the Metavir score system (F0 to F4). We also studied patients for whom paired biopsies (4–9 years) were available and progressed from mild fibrosis (F1) to significant fibrosis (F2 to F4). We selected miR-20a, miR-92a, miR-122 because of their implication in liver fibrosis. Their expression was assessed by RT-qPCR.
Results: Transaminases were higher in patients with severe fibrosis compared to patients with mild and moderate fibrosis ($p < 0.001$). In serums, while circulating mir-122 was correlated with transaminases ($p < 0.02$), no such association was found for mir-92a and mir-20a. Circulating miR-122 was increased in F3–F4 patients compared to F1–F2, but no difference was observed for mir-92a and mir-20a. No association was found between necroinflammatory activity and circulating miR-20a, miR-92a, and miR-122. In liver biopsies, a higher expression of hepatic miR-122 ($p < 0.0001$), miR-92a ($p = 0.026$) and miR-20a ($p = 0.024$) was observed in patients with mild (F1) and moderate fibrosis (F2) compared to those with severe fibrosis (F3–F4). This result was confirmed in patients whose fibrosis stage increased from mild to moderate fibrosis.
Conclusions: The expression of hepatic miR-122, miR-92a and miR-20a was higher in patients with mild and moderate fibrosis as compared to those with more advanced fibrosis. However, the expression of those 3 miRNAs was not different in the serum. Only miR-122 was associated with transaminases and no association was found with necro-inflammation.

P0524

IMPACT OF NUMBER OF MEASUREMENTS ON DIAGNOSTIC PERFORMANCE OF REAL TIME SHEAR-WAVE ELASTOGRAPHY: A BIOPSY-CONTROLLED STUDY

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Background and Aims: Real time shear-wave elastography (SWE) is a novel technique with excellent diagnostic accuracy for liver fibrosis and cirrhosis. However, there is no consensus on how many measurements should be obtained to gain the best diagnostic accuracy, or whether to use the mean or median when reporting results. To assess the most optimal reporting of SWE results, we compared diagnostic accuracies of seven methods of reporting, using liver biopsy as gold standard.
Methods: During 6 months we consecutively recruited patients from an ongoing biopsy-controlled study on the performance of elastography for the diagnosis of liver fibrosis. Five SWE measurements, transient elastography (TE) and liver biopsy were performed on the same day. We applied two SWE quality criteria: Q-box ≥15 mm in diameter and a standard deviation of less than 30% of the mean. SWE results were reported by seven methods: A: one measurement, B: the mean of two, C: three, D: four and E: five measurements, and F: the median of three or G: five measurements. We used AUROC comparisons to compare the diagnostic accuracies of the seven methods. We also assessed which reporting method best correlated with TE.
Results: Of 71 patients evaluated, five valid SWE measurements were obtained in 67 patients (failure rate 6%). The majority had alcoholic liver disease (51 ALD, 16 HCV) and 61% were men. Our cohort represented a broad spectrum of liver disease (METAVIR F0/1/2/3/4 = 16/16/11/5/19). The mean liver stiffness was 14.0 ± 10.8 kPa with a mean difference between the five measurements of 3.7 ± 4.2 kPa (range 0.3–26). The mean difference between the seven reporting methods were 1.6 ± 1.7 kPa (range 0.2–8.6).
The diagnostic accuracy for significant fibrosis (≥F2) was excellent when using any of the seven reporting methods. AUROCs ranged from 0.961 (A) to 0.942 (G). There was no difference in diagnostic

accuracy between the seven methods (P = 0.58). The diagnostic accuracy for cirrhosis (F4) was also excellent, without differences across methods (P = 0.87), and AUROCs from 0.952 (D) to 0.942 (A). All seven SWE reporting methods correlated highly with TE [Spearman's rho, range 0.91 (D) to 0.89 (G)], without any one reporting method correlating better with TE than the others.
Conclusions: The diagnostic performance of SWE does not increase with the number of measurements. Applying a set of quality criteria ensure a low variability between measurements. One measurement is enough to gain excellent diagnostic accuracy for both significant fibrosis and cirrhosis.

P0525
N-ACETYLATED ALPHA SMOOTH MUSCLE ACTIN LEVELS ARE INCREASED IN HEPATIC FIBROSIS BUT DECREASED IN HEPATOCELLULAR CARCINOMA

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Background and Aims: Alpha Smooth Muscle Actin (α-SMA) is upregulated together with extracellular matrix (ECM) during activation of Hepatic Stellate Cells (HSCs) in fibrosis. Histone deacetylase (HDAC) remove acetylations and regulate the expression of genes, which is associated with cancers. There is a close relationship between cirrhosis and hepatocellular carcinoma (HCC), and markers enabling identification of patients in risk of developing HCC with cirrhosis is a major unmet clinical need. We developed an ELISA for the assessment of acetylated α-SMA (Ac-α-SMA) in serum. The objective was to investigate the ability of this marker to non-invasively diagnose hepatic fibrosis and assess the influence of HDAC in hepatocellular carcinoma (HCC).
Methods: We generated a monoclonal antibody towards the N-terminus of α-SMA containing acetylation of the third amino acid, N-acetyl-glutamic acid. A competitive ELISA was developed and tested for robustness and the presence of acetylation of the third amino acid on the synthetic peptides was analyzed by mass spectrometry. The biological relevance of the Ac-α-SMA marker was tested in serum and tissue cultures from a Carbon tetrachloride (CCl_4) liver fibrosis rat model and a diethylnitrosamine (DENA) cancer rat model.
Results: Mass spectrometry analysis confirmed the presence of N-acetylation of the peptide compared to a non-acetylated α-SMA peptide. The assay was found to be technical robust including intra- and inter-variations below 10% and 15%, respectively, and showed no reactivity towards the non-acetylated peptide. Ac-α-SMA was significantly increased in serum from CCl_4 induced rats compared to healthy controls (p = 0.002) whereas the level of Ac-α-SMA was significantly decreased in DENA rats compared to healthy controls (p = 0.002). Similar results were found for western blot analysis showing increased expression of Ac-α-SMA in CCl_4 livers compared to controls.
Conclusions: We developed a technical robust serum marker specific for Ac-α-SMA. The level of Ac-α-SMA was increased in hepatic fibrosis, and decreased in HCC. This suggests that acetylations could be the discriminating factor in identification of those patients who progress to HCC.

P0526
ACCURACY OF POINT SHEAR WAVE ELASTOGRAPHY TECHNIQUE (ELASTPQ) IN THE NON-INVASIVE ASSESSMENT OF LIVER FIBROSIS IN VARIOUS LIVER DISEASES

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Background and Aims: Point shear wave elastography (PSWE) is a novel non-invasive technique that assesses liver fibrosis by measuring liver stiffness (in kPa) with few studies published so far. The purpose of this study was to determine the efficacy and the feasibility for the assessment of hepatic fibrosis as compared with the histological grade in patients undergoing liver biopsy (LB) for various etiologies.
Methods: Consecutive patients scheduled for LB were studied by using the iU22 Philips ultrasound system with ElastPQ technique. The correlations between laboratory findings, liver stiffness and the Metavir score were analyzed using Spearman correlation and ROC curve analyses were performed to calculate AUC for F > 2, F > 3, and F = 4.
Results: We enrolled 172 patients (113/59 males/females) who underwent LB for viral or non-viral chronic hepatitis (HCV 46%; NASH 23%; AIH/PBC 14%; other 17%). Liver stiffness measurements performed on the right lobe were reliable in all cases but one (due to morbid obesity). After univariate and multiple regression analysis PSWE showed a strong correlation with the fibrosis stage; no correlation was found with the degree of necroinflammation or steatosis. Mean kPa values in the whole cohort were 3.62 (range 2.81–4.69) for F0, 4.94 (range 2.81–9.68) for F1, 7.74 (4.16–20.79) for F2, 12.32 (6.25–19.78) for F3 and 23.32 (18.91–32.40) for F4 in the right lobe. AUROCs were 0.92 (±0.17), 0.97 (±0.11) and 0.98 (±0.03), when comparing F0–F1 vs F2–F4, F0–F2 vs F3–F4 and F0–3 vs F4, respectively. When analyzing PSWE values according to different etiologies, AUROCs were 0.93 (±0.31) and 0.97 (±0.29) for F > 2, 0.99 (±0.03) and 0.97 (±0.30) for F > 3, 0.99 (±0.03) and 0.99 (±0.03) for F > 4 in HCV and NASH patients, respectively.
Conclusions: PSWE with ElastPQ appears to be a useful tool for non-invasive evaluation of fibrosis not only in patients with viral chronic hepatitis, but also for patients with different liver diseases. In order to validate such a non-invasive technique these findings need to be confirmed in larger studies.

P0527
PERFORMANCE OF FIBROSCAN XL PROBE WITH ADAPTED MEASUREMENT DEPTH FOR LIVER STIFFNESS MEASUREMENT IN MORBIDLY OBESE PATIENTS

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Background and Aims: The XL probe of transient elastography (FibroScan®) was recently developed to evaluate liver stiffness measurements (LSM) in overweight and obese patients. However overlying fat layers in morbidly obese patients (BMI ≥40 kg/m^2) are much thicker and present a major inter-individual variability and thus could lead to an overestimation of stiffness. In this study, we evaluated the diagnostic performance of XL probe after adapting LSM depths in morbidly obese candidates for bariatric surgery.
Methods: From 09/2010 to 04/2014, 119 morbidly obese patients (69.7% female; age 41±11 years; BMI 46.9±7.3 kg/m^2) candidates for bariatric surgery were recruited and underwent LSM using

XL probe before surgery. Per-operative liver biopsy (LB) samples were collected and evaluated by a single anatomo-pathologist using Kleiner's classification for fibrosis. Valid LSM was considered for who had at least 5 valid measurements. Probe performance for diagnosing ≥F2 and ≥F3 fibrosis versus biopsy were examined using AUROC.

Results: In 94 patients who had valid LSM, 26% had significant fibrosis (14% F2, 10% F3, 3% F4). AUROC of standard XL LSM (35–75 mm) for significant fibrosis (F≥2) was 0.84 (0.75–0.94), for F ≥3 was 0.84 (0.71–0.97). To recalculate LSM, three adapted measurement depths were selected according to fat layer: 35–75 mm, 40–75 mm and 45–80 mm. AUROC of adapted LSM for significant fibrosis (F≥2) was 0.85 (0.75–0.94); AUROC for F≥3 was 0.92 (0.83–1). 10 patients who had overestimated LSM using standard depth were significantly more obese (BMI 51.8±6.0 kg/m^2 versus 44.5±5.9 kg/m^2, $p = 0.001$).

Conclusions: FibroScan allows the early diagnosis of significant fibrosis in morbidly obese patients before bariatric surgery. This study demonstrates that fat layer thickness superior to 40 mm in such patients requires an adaptation of the LSM calculation but the measurements are feasible and accurate. FibroScan appears as a reliable and noninvasive tool for screening chronic liver disease severity in morbidly obese patients. Further studies are needed to confirm these results.

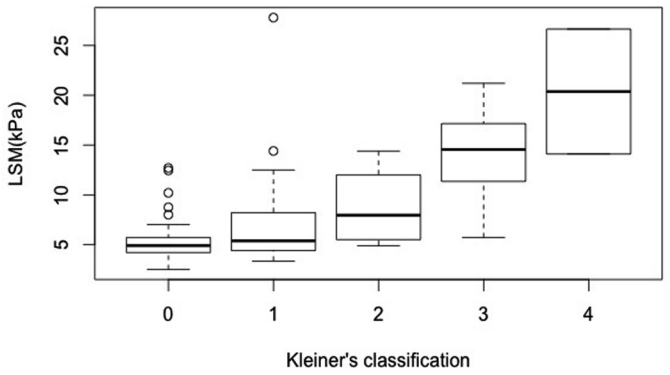

Figure: Boxplot of adapted LSM of FibroScan XL.

P0528
TRANSIENT ELASTOGRAPHY IS SAFE IN PATIENTS WITH PACEMAKERS AND IMPLANTABLE CARDIOVERTER-DEFIBRILLATORS

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Background and Aims: Transient elastography (TE) is widely used for non-invasive assessment of liver fibrosis and is recommended by EASL Clinical Practice Guidelines for chronic hepatitis C. However, TE is not accredited in patients with pacemaker (PM) and implantable cardioverter-defibrillator (ICD) devices, since side effects on PM and ICD are unknown. Therefore, studies in these patients are missing. The aim of the present study was to evaluate whether liver stiffness measurement (TE) using FibroScan® effects safety and function of such devices.

Methods: In a prospective study between January 2012 and October 2014 patients with PM or ICD receiving TE underwent two PM/ICD check-ups, one immediately before and one after TE. At least 10 TE-measurements were performed with the FibroScan® M-Probe (3.5 MHz; shear wave frequency 50 Hz; depth of measurement 25–65 mm) using the FibroScan® 502 touch device (Echosens, Paris, France). During PM/ICD check-ups stimulation thresholds, electrode impedance and sensing were recorded and changes after TE-performance as compared to the check prior to TE were documented and analyzed.

Results: Thirty-four patients [82% male; mean age 73±10 years; median body mass index 27.8 kg/m^2 (range 17.8–43.0 kg/m^2)] with PMs (n = 16) or ICDs (n = 18) were enrolled in the present study. In none of these 34 patients changes in stimulation thresholds, electrode impedance and sensing of PM or ICD were detected. Furthermore, none of the probed devices recorded any cardiac events during TE-examination. All patients reported well-being throughout the whole study period.

Conclusions: Our findings support the assumption that transient elastography can be performed safely in patients with implanted pacemakers and implantable cardioverter-defibrillators.

Viral hepatitis: Hepatitis B & D – a. Experimental

P0529
ANTI CAPSID DRUGS HAP12 AND AT130 TARGET HBV CORE PROTEIN NUCLEAR FUNCTIONS

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Background and Aims: HBV Core protein (Cp) represents an attractive new therapeutic target for HBV chronic infection. In addition to their role in capsid assembly, pgRNA packaging and reverse transcription, Cp has been shown to bind the nuclear cccDNA mini-chromosome as well as a number of cellular genes promoters. Several compounds that target Cp and HBV capsids assembly, including the Hetero-aryl-dihydropyrimidines (HAPs) and the phenyl-propenamide derivatives AT61 and AT130, have been shown to inhibit HBV replication in vitro and in vivo. HAPs and AT130 enhance the rate and the extent of Cp assembly leading to non-functional capsids and, at high concentration, stabilize preferentially non-capsid polymers of Cp. Here we investigated the ability of the Core protein Assembly Modulators (CaMPs) HAP12 and AT130 to affect both nuclear (cccDNA structure and transcription) and cytoplasmic (capsid maturation and replication) Cp functions as part of their antiviral activity against HBV.

Methods: HAP12 and AT130 effects on capsid-associated HBV-DNA (TaqMan real-time PCR), cccDNA (TaqMan real-time PCR) and pgRNA levels (quantitative real-time PCR with specific primers), were assessed in: (a) HBV-infected NTCP-HepG2 cells; (b) AD38 inducible HBV stable cell line. Recruitment of HBc and histone modifications on the viral minichromosome were assessed using the cccDNA ChIP assay in AD38 cells.

Results: CaMPs treatments started at day 6 post-infection (NTCP-HepG2) or day 6 post-induction (AD38 tet-off cells) resulted in a very strong inhibition of HBV replication (>95%) and a significant but incomplete reduction of the stable cccDNA pool. A strong effect on cccDNA-dependent HBeAg production (AD38 tet-off) and pgRNA transcription (AD38 tet-off / tet-on and NTCP-HepG2 infected cells) was also demonstrated. The ability HAP12 to target cccDNA transcription was confirmed by the reduced cccDNA-bound H3 histone acetylation and the descreased HBc occupancy on the

cccDNA in induced AD38 cells. Importantly, when CaMPs treatment was started during infection, cccDNA formation/accumulation was completely inhibited (>95%) and viral replication was blunted.
Conclusions: Anti-capsid compounds (CpAMs) have an impact on Cp nuclear functions at multiple levels: block of new cccDNA formation / accumulation, reduction of an established cccDNA pool and inhibition of HBc occupancy and histone acetylation on the cccDNA that translate into a reduced pgRNA transcription.

P0530
MYRCLUDEX-B INHIBITS ESTABLISHMENT OF HDV SUPER-INFECTION IN HBV INFECTED MICE AND REDUCES HDV VIREMIA IN STABLY HBV/HDV CO-INFECTED MICE

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Background and Aims: 15 million people are infected with the hepatitis Delta virus (HDV) worldwide. Therapeutic strategies specifically targeting HDV infection are not available but urgently needed. We previously demonstrated prevention of de novo HBV/HDV co-infection using the entry inhibitor Myrcludex-B in naïve human liver-chimeric uPA/SCID mice (Lütgehetmann, Hepatology 2012). Aim of the study was to assess (I) whether treatment with Myrcludex-B can hinder infection establishment and spreading of HDV also in humanized mice already infected with HBV, and (II) whether long-term entry inhibition can affect viremia levels in stably HBV/HDV co-infected mice.
Methods: Humanized uPA/SCID mice were first stably infected with HBV, then super-infected with HDV and treated with Myrcludex-B (2 mg/kg; daily) 2 days before until 5 weeks after HDV inoculation (I), while stably HBV/HDV co-infected mice received Myrcludex-B daily for 9 weeks (II). Viral loads were quantified in serum and liver by qRT-PCR and visualized by immunohistochemistry.
Results: (I) In HBV-infected mice, which received Myrcludex-B during the first 5 weeks of HDV super-infection, HDV viremia, intrahepatic HDV RNA and HDAg remained below detection levels. However, one mouse showed development of HDV viremia 3 weeks after treatment cessation, demonstrating that Myrcludex-B strongly hindered but did not completely abrogate establishment of HDV infection in humanized mice. (II) In stably HBV/HDV co-infected mice, 9 weeks of Myrcludex-B treatment induced 1-log reduction of HDV, while HBV viremia was similar in untreated and treated mice. However, no significant intrahepatic viral changes could be determined by comparing treated and untreated animals. The ratio of HDV RNA quasi-species encoding for the small and large HDAg was also maintained after 9 weeks of Myrcludex-B administration.
Conclusions: Myrcludex-B significantly inhibited HDV infection establishment also in the presence of a productive HBV infection. However, continuous drug administration was necessary to prevent HDV spreading from the very few initially infected human hepatocytes. Long-term treatment of HBV/HDV co-infected mice with the entry inhibitor was needed to detect HDV viremia decrease. The high infection efficiency and great survival capacities of HDV shown in this study highlight the difficulties encountered in treating HBV/HDV co-infected patients.

P0531
TRANSGENIC MICE CARRYING HEPATITIS B VIRUS PRE-S/S GENE CONTAINING THE rtA181T/sW172* MUTATION DEVELOP HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatitis B virus carrying the rtA181T/sW172* mutation conferred cross-resistance to adefovir and lamivudine. Cell-based and epidemiological studies indicated that hepatitis B virus pre-S/S proteins carrying this mutation had an increased oncogenic potential. Here we created transgenic mice models to study this issue.
Methods: Transgenic mice were generated by transfer of hepatitis B virus pre-S/S gene (genotype A) together with its own promoter into C57B6 mice.
Results: Four lines of transgenic mice were created. Two of them carried wild type pre-S/S gene and produced high and low levels of HBsAg (TgWT-H and L). The other two carried mutant type pre-S/S gene containing the rtA181T/sW172* mutation (TgSW172-H and L). Western blot/IHC analysis detected high and low levels of intrahepatic truncated pre-S/S proteins respectively in TgSW172-H and L mice. The former had detectable low level of HBsAg in the serum, while the latter was negative for serum HBsAg (secretion failure). All mice were sacrificed 18 months after birth. None of the TgWT mice developed hepatocellular carcinoma (HCC), whereas 6/26 (23.1%) TgSW172-H and 2/24 (8.3%) TgSW172-L mice developed HCC. Molecular analysis of liver tissues revealed significantly increased expression of GPR78 and phosphorylated ERK1 in TgSW172 mice versus TgWT mice; and decreased expression of Bcl-XL in TgSW172-H mice versus TgSW172-L and TgWT mice. Higher proportion of apoptotic cells assessed by TUNEL assay was found in TgSW172-H than in TgWT mice, but both showed increased cyclin E levels, suggesting increased hepatocyte turnover and regeneration. Combined analysis of cDNA microarray and microRNA array showed reduced expression of the CUB and Sushi multiple domains 3 (CSMD3) protein, a putative tumor suppressor, in TgSW172 but not TgWT mice.
Conclusions: Transgenic mice experiments confirmed that the pre-S/S gene carrying rtA181T/sW172* mutation had an increased oncogenic potential in development of liver cancer. Increased ER stress with more rapid hepatocyte turnover as well as decreased expression of CSMD3 contributed in part to hepatocarginogenesis.

P0532
HBx–DLEU2 lncRNA COMPLEX AFFECTS TRANSCRIPTION OF NEW TARGET PROMOTERS

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Background and Aims: HBx affects HBV mini-chromosome transcription, by preventing HDACs and PMRT1 recruitment on the cccDNA, and cellular genes expression, by favoring the recruitment of positive and negative chromatin modifying enzymes. A ChIPSeq analysis of HBx genome wide recruitment identified 39 long non coding RNAs (lncRNAs), including DLEU2, as direct HBx transcriptional targets in HBV replicating cells. DLEU2 lncRNA overlaps the first exon of the TRIM13 gene in the opposite orientation and the pri-Mir of the miR-15a/16–1 cluster. Up-regulation of specific DLEU2 splicing variants correlates with tumors. TRIM13 induces autophagy and increase ectopic levels of p53.

Methods: Anti-HBx ChIP were performed in HepG2 cells replicating wt and HBx-mt HBV. HBx ChIPed DNA and lncRNA/gene expression levels were analysed by TaqMan RT-PCR using specific primers. Specific LNA™ longRNA GapmeRs (Exiqon) were used for highly efficient inhibition of DLEU2 lncRNA function. I-Tasser was used for *ab initio* prediction of HBx protein structure. RNA secondary and tertiary structures were predicted with the *RNAfold* and *3dRNA* softwares, respectivly. Protein-RNA interactions were calculated by *Hex*.
Results: We show that HBx binding to the DLEU2 promoter leads to increased H4 acetylation, a different DLEU2 splicing profile, down-regulation of miR-15a/16–1 cluster and up-regulation of the antisense autophagic gene TRIM13, in the absence of HBx binding to the TRIM13 promoter. GapmeR-mediated selective degradation of DLEU2 RNA results in reduced TRIM13 promoter H4 acetylation and a ~50% reduction of TRIM13 expression in HBV replicating cells. These results directly link DLEU2 RNA species with TRIM13 transcriptional regulation in the presence of HBx. The interaction between HBx and DLEU2 was confirmed by RIP (RNA Immune Precipitation) experiments. We further characterized the HBx-DLEU2 complex *in silico* by coupling *ab initio* modeling of HBx protein and DLEU2 RNA tertiary structures and we constructed a docking model of DLEU2-HBV complex. Finally, we found that DLEU2 inactivation has a profound impact on pgRNA transcription, suggesting a functional relevance of DLEU2-HBx interaction for HBV replication.
Conclusions: HBx targets the DLEU2 promoter leading to a different DLEU2 splicing profile and its binding to DLEU2 RNA species affects cellular genes transcription and HBV replication.

P0533
THE EFFICACY OF ZINC FINGER ANTIVIRAL PROTEIN AGAINST HEPATITIS B VIRUS TRANSCRIPTION AND REPLICATION IN TRANSGENIC MOUSE MODEL

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Background and Aims: The zinc finger antiviral protein (ZAP) is a mammalian host restriction factor, and it could inhibit HBV RNA synthesis in vitro experiments. However, the role of ZAP against HBV in vivo environment is unclear. This study aimed to investigate whether ZAP could act against HBV transcription and replication in ZAP tansgenic mouse model.
Methods: HBV-replication-competent plasmid pHBV4.1 was transferred to ZAP transgenic ICR mice via the tail vein, using a hydrodynamic in vivo transfection procedure. HBV RNA and HBV DNA replication intermediates in the liver were respectively analyzed by Northern blotting and Southern blotting. The expression of hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) in the liver tissue was detected by immunohistochemical staining.
Results: As compared to control ICR mouse, the level of HBV RNA in ZAP transgenic mouse liver tissue was only decreased by 8.4%; while the level of HBV DNA replication intermediates was decreased by 82%. In addition, the expression levels of HBsAg and HBcAg in ZAP transgenic mouse liver were both significantly less than that of control ICR mouse.
Conclusions: Our findings suggest that ZAP could inhibit HBV replication in vivo in mice, which offers a new target for anti-HBV drug development.

P0534
HEPATITIS E VIRUS RNA REPLICATION IS ENSURED BY UNPROCESSED ORF1 PROTEIN

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Background and Aims: Hepatitis E virus (HEV) infection is believed to be the most common cause of acute hepatitis and jaundice in the world. HEV is a non-enveloped positive-strand RNA virus whose genome encodes 3 open reading frames, namely ORF1, ORF2 and ORF3. The aim of this study was to clarify whether the HEV ORF1 protein, which harbors the viral genome replication functions, is processed into distinct functional proteins.
Methods: ORF1 from HEV genotypes 1 and 3 was expressed in different experimental systems, including (i) a wheat germ-based cell-free expression system, (ii) a T7 polymerase-based cellular expression system, (iii) newly established human cell lines inducibly expressing HEV ORF1, as well as (iv) selectable subgenomic HEV replicons derived from the Kernow-C1 strain, followed by immunoblot and immunofluorescence analyses using antibodies directed against different ORF1 domains, i.e. the methyltransferase, helicase and RNA-dependent RNA polymerase.
Results: The product of HEV ORF1 was detected only as single a protein of ~190 kDa in the four experimental systems investigated, including in a context of authentic viral RNA replication. In addition, the different ORF1 domains perfectly colocalized at an as yet undefined cellular compartment. Collectively, these results indicate that no or only very inefficient processing of the polyprotein encoded by ORF1 occurs during viral RNA replication.
Conclusions: Our results indicate that HEV RNA replication is ensured by unprocessed ORF1 protein. Efforts are ongoing to validate these findings in a fully infectious system. Our findings yield a number of intriguing questions regarding the functional organization, structure and cell biology of the polyprotein encoded by HEV ORF1.

P0535
DIRECT ANTIVIRAL EFFECTS OF VARIOUS PATTERN RECOGNITION RECEPTOR (PRR) AGONISTS IN HBV-REPLICATING HEPATOCYTES

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Background and Aims: Current therapies for chronic hepatitis B virus (HBV) infection are effective at suppressing viral replication and improve long-term outcome, but have only low rates of HBsAg loss and anti-HBs seroconversion. There is an urgent need to identify new antiviral therapies to achieve functional cure and so decrease the risk of end-stage liver disease. Studies with the oral TLR7 agonist GS-9620 in animal models of chronic hepatitis B (CHB) have recently highlighted the potential of therapeutic immune modulation by small molecule agonists of innate immunity. To further explore the potential of PRR agonists to induce efficient and durable immune control against HBV, we tested the direct anti-HBV effect of different PRR agonists in cell culture models of natural infection.
Methods: Primary human hepatocytes (PHH) and differentiated HepaRG cells (dHepaRG), which are both innate immune competent cells, were infected with HBV for 7 days, and then treated with

various concentrations of different PRR agonists for 7 days. Antiviral activity was evaluated by quantifying HBeAg and HBsAg secretion (ELISA), HBV RNA (qRT-PCR) and total intracellular HBV DNA and cccDNA (qPCR), and toxicity measured by MTS assay and ApoB ELISA. Cytokine production was evaluated by ELISA 24 hours (h) post-stimulation.

Results: The cytokines IP-10 and/or IL-6 were secreted by both PHH and dHepaRG 24 h after stimulation with TLR1/2, TLR3, TLR4, TLR5, TLR2/6, RIG-I/MDA5, and AIM2 agonists, which correlated with a strong and dose-dependent antiviral effect in the absence of toxicity. The maximal antiviral effect was induced by Pam3CSK4 (i.e. TLR1/2-L), which induced sustained reduction in all viral parameters, including levels of cccDNA. Stimulation with TLR7, TLR8 and TLR9 agonists did not significantly induce IP-10 or IL-6 production and, with the exception of imiquimod, did not inhibit HBV replication. Interestingly, the antiviral response induced by imiquimod was independent of innate immune activation, suggesting an additional mode of action that should be further investigated.

Conclusions: Our data highlight the potential of direct innate immunity activation in hepatocytes in the control of HBV replication, and support the recent interest in the development of PRR-based antiviral strategies against HBV. In addition, our data suggest that elucidation of the mode of antiviral action of imiquimod may lead to the identification of a novel target for the treatment of CHB.

P0536
OSTEOPONTIN IS INVOLVED IN CHRONIC HBV INFECTION AND ENHANCES HBV REPLICATION AND HBsAg SECRETION

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Background and Aims: Hepatitis B virus (HBV) requires host cellular machinery such as cyclophilins to support its ongoing propagation (Phillips et al, Gastroenterology 2014), these host proteins represent ideal candidates for therapeutic interventions as they are generally expected to have a lower frequency of drug-resistance and antiviral efficacy across genotypes. We have also recently described a role for the host protein Osteopontin (OPN), a pro-fibrogenic downstream effector of the Hedgehog pathway, in enhancing HCV replication (Choi et al, Clinical Sciences 2014). Whilst increased levels of blood OPN have similarly been reported in chronic Hepatitis B (CHB), its role in viral replication remains unknown. This study aimed to evaluate the role of OPN in HBV replication, HBsAg secretion and HBV-driven liver injury.

Methods: Stably (HepG2215), transiently (HUH-7) transfected and infected (HepaRG) cell lines, producing full HBV virions and HBsAg particles were cultured over 72 h in the presence/absence of several concentrations of recombinant OPN (recOPN). In addition, secreted OPN was neutralized using OPN-specific aptamers. Cells and supernatants were harvested at baseline, 24, 48 and 72 hours. Intracellular OPN mRNA and HBV-DNA levels were quantitated by Real-Time qPCR. HBsAg levels were measured by ELISA. OPN levels were also measured by ELISA in cell culture supernatants and in sera of controls and HBeAg(+) CHB patients who were either treatment naive or treated with potent antiviral agents. In addition, expression of OPN was assessed in explanted livers from healthy and HBV-cirrhotic patients.

Results: Serum levels and intrahepatic expression of OPN were significantly increased in CHB patients compared to controls (up to 7 fold; $p < 0.05$). In vitro, mRNA and protein levels of OPN were highly correlated with intracellular HBV-DNA ($r = 0.858; p < 0.001 / r = 0.997; p = 0.003$), secreted HBV-DNA ($r = 0.968; p < 0.001 / r = 0.818; p = 0.047$) and secreted HBsAg ($r = 0.738; p = 0.001 / r = 0.89; p = 0.018$). The relationship between OPN and HBV replication was further confirmed following treatment with recOPN which showed a significant increase in intracellular and secreted HBV-DNA by an additional 1.3 Log10 copies/mL and amplified HBsAg secretion rates by 2 fold.

Conclusions: These data confirm that OPN is upregulated in the blood and livers of CHB patients. We also show that OPN directly augments HBV replication, thus identifying a novel therapeutic target.

P0537
DATING THE ORIGIN OF HBV USING FULL-LENGTH SEQUENCES

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Background and Aims: The origin of HBV has been debated. Our previous analyses based on the overlapping S/P region suggested that HBV has followed major expansion of modern humans, with its origin to be estimated 22,000–47,000 years ago [1]. Our aim was to test the hypothesis of co-expansion between HBV and humans and to propose a new approach for dating based on full-length genomic sequences.

Methods: Analyses were performed on 105 HBV sequences from all major genotypes. We initially tested the co-expansion hypothesis performing a Bayesian molecular clock analysis calibrated at the origin of the F/H genotypes from Amerindians. To date the origin and evolution of HBV we use five calibration points at the origin of F/H genotypes, D_4, C_3 and B_6 from indigenous populations in Pacific and Arctic, respectively, and A_5 from Haiti. We translated anthropological, genetic and archaeological time estimates into statistical distributions, taking uncertainty of those estimates into account, to be used as calibration densities on an HBV phylogeny estimated with ML. Bayesian dating analyses were performed using the MCMCTree program. The analyses were based on three partitions across the genome.

Results: The molecular clock analysis using a calibration on the most recent common ancestor (MRCA) of F and H genotypes estimated the origins of C_3 and D_4 at 5,910 and 10,558 years ago, which coincide with genetic estimates of the timing of human population movements (5,100–8,100 and 6,200–12,000 years, respectively). The estimated time for the origin of HBV was 34,103 (95% HPD CI: 27,623–41,347) years ago, coinciding with the origin of modern non-African humans. Moreover, the time estimates of HBV lineages had significantly narrower credibility intervals than in a previous analysis of the overlapping S/P region [1]. The substitution rates for the different partitions were estimated at 2.6×10^{-6}, 4.4×10^{-6} and 4.4×10^{-6} substitutions/site/year.

Conclusions: Our study, using for the first time full-length sequences, provides an estimate of the timescale of HBV epidemic that coincides with dates of migrations of modern humans. The hypothesis of co-expansion of the pathogen and the human host was supported by our findings. Our study provides a new approach for dating HBV based on the most informative full-genomic HBV alignment.

Reference(s)

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P0538

TRACING HBV GENETIC HISTORY IN THE ARCTIC: EVIDENCE FOR A CENTRAL ASIAN HBV ORIGIN IN GREENLAND

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Background and Aims: HBV genotypes have a distinctive distribution in the Arctic: B_6 is unique to Inuit populations of North America and Greenland; D_3 and D_4 circulate primarily among the First Nations (Na-Dene) population in northern Canada and D_1/D_2 is found among Inuit in Greenland. Our aim was to estimate the origin and dispersal of HBV infection in indigenous Arctic populations and understand better the global mobility of the HBV epidemic.
Methods: We used a phylogenetic approach to study the dispersal of HBV genotype D (*N*=81 sequences) using all sequences available (*N* = 2,965) in the overlapping P/S region. To estimate the date of HBV origin we performed a Bayesian dating analysis using 128 sequences and five calibration points at the origin of F/H genotypes, D_4, C_3, B_6 and A_5. Based on anthropological, genetic and archaeological time estimates of human migrations, we calibrated the HBV molecular clock. We estimated the timescale of HBV evolution using the program MCMCTree.
Results: Phylogenetic analyses suggest that subgenoypes D_4 and D_1/D_2 from Canada and Greenland are monophyletic, whereas D_3 strains from Na Dene are dispersed within the D_3subgenotype. The D_1/D_2sequences were more closely related to viral strains from Central Asia (Turkey, India), but their exact origin is not evident. The time of origin of B_6 was estimated at 5,904 (95%HPD CI: 5,180–6,470), of D_4 in the Arctic at 2,948 (1,120–5,510) and of D_1/D_2 4,104 (1,860–5,880) years ago. Subgenotype D_4 (tMRCA: 6,579; 95%HPD: 4,650–11,150) formed two major clades: one from Qinghai-Tibet (tMRCA: 3,393; 95%HPD: 1,170–5,930) and another from the Pacific, the Arctic and the Americas. This suggests that HBV D_4 has followed a long dispersal through Central Asia in Tibet, and then took two distinct routes in Remote Oceania and North Eastern Asia from where it migrated to Americas.
Conclusions: Our analysis, based on one of the largest HBV datasets from Arctic indigenous populations, provided evidence that in addition to B_6, D_4 and D_1/D_2 are inherent to these populations. In contrast, D_3 was the result of multiple introductions. The distinct ages of HBV subgenotypes could suggest that introduction of HBV in the Arctic might have occurred at different time periods. Notably our study provides evidence that D_1/D_2 found at low prevalence in Greenland probably originated from Central Asia, where D_1/D_2 is circulating. To our knowledge this is one of the few studies showing genetic evidence for a Central Asian-Greenlandic HBV association.

P0539

EXPRESSION PROFILE OF Tim-3 INDUCED BY MULTIPLE CYTOKINES AND ANTIGENS IN PATIENTS WITH CHRONIC HEPATITIS B

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Background and Aims: Previous studies have demonstrated HIV-1 could not directly induce the expression of T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) on $CD4^+$ and $CD8^+$T cells, However, Tim-3 could be markedly enhanced by the common gamma-chain (γc) cytokines. Thus, the aim of the study was to investigate whether the expression of Tim-3 on the $CD4^+$ and $CD8^+$ T cells in HBV infection was closely related to the gamma-chain cytokines and HBV associated antigens.
Methods: Peripheral blood mononuclear cells was obtained from chronic hepatitis B (CHB) patients (n = 108) and healthy volunteers (n = 30). PBMCs were stimulated by HBV Ag, HBV Pep or gamma-chain cytokines, respectively. The expression of Tim-3 on peripheral $CD4^+$ and $CD8^+$ T cells was determined by flow cytometry.
Results: Under the stimulation of HBV Ag and HBV Pep, The Tim-3 expression by HBV Pep stimulated was elevated on peripheral $CD4^+$ and $CD8^+$ T cells from CHB patients compared to those from the healthy controls. After the gamma-chain cytokines stimulation, the increased-expression of TIM-3 was also detected on peripheral $CD4^+$ and $CD8^+$ T cells of CHB patients compared to that of the healthy controls. Given the combined stimulation of the gamma-chain cytokines and HBV Pep, there are also a enhanced expression of Tim-3, as well as the results of their alone stimulation.
Conclusions: The current results suggest that the HBV Ag, HBV Pep, and the gamma-chain cytokines could induce the over-expression of Tim-3 in the HBV patients, which may indicate that the inhibition of these cytokines can not only decrease the expression of Tim-3, but also may enhance the cytotoxic function of $CD8^+$T cells in patients with CHB.

P0540

THE SUBSTITUTION AT rt269 IN HEPATITIS B VIRUS POLYMERASE IS ASSOCIATED WITH THE MULTI-DRUG RESISTANCE AS A COMPENSATORY MUTATION

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Background and Aims: The emergence of compensatory mutation in polymerase gene of drug resistant hepatitis B virus (HBV) is associated with treatment failure. We previously identified a multi-drug resistant HBV mutant which showed resistant to lamivudine (LAM), clevudine (CLV), and entecavir (ETV) with strong replication ability. The aim of this study was to identify the previous unknown compensatory mutation and to search the clinical relevance of this mutation during antiviral therapy.
Methods: In vitro mutational analysis and drug susceptibility assay were performed to characterize the function of rtL269I mutation.
Results: The point mutation of rtL269I showed about 2- to 7-fold higher replication ability in wt or YMDD mutation back-bone regardless of drug treatment. The rtL269I mutation by itsefl did not show the resistance to LMV, CLV, ETV, adefovir (ADV), and tenofovir (TDF) treatment. However, when the rtL269I was combined with YMDD mutation, it enhanced the replication ability by several folds under LMV or ETV treatment. Molecular modeling study predicted that rtL269I mutation may affect the negative antiviral action of nucleos(t)ide drugs and incoming dNTP binding.
To investigate the clinical relevance of rtL269I mutation, we analyzed a total of 22 patients who showed suboptimal response or treatment failure during antiviral monotherapy and found that 16 patients had the YMDD mutation. Among them, 7 (43.8%) patients harbored the rtL269I mutation. We further analyzed so-called the "difficult-to-treat" cases who experienced the complex history of antiviral therapy. Among 14 "difficult-to-treat" patients, the 13 patients had rtL269I mutation during the follow-up.
Conclusions: Collectively, our study suggests that the substitution at rt269 in HBV polymerase is associated with the multi-drug

resistance and may serve as a novel compensatory mutation for replication defect of multi-drug resistant HBV.

P0541

SMALL INTESTINAL PERMEABILITY IS ELEVATED IN CHRONIC VIRAL HEPATITIS PRIOR TO THE DEVELOPMENT OF CIRRHOSIS, AND IS ASSOCIATED WITH THE DEGREE OF FIBROSIS

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Background and Aims: Small intestinal permeability may play a role in fibrosis progression in chronic liver disease. However portal hypertension itself is likely to contribute to increased permeability measurements. Few data exist in patients with chronic viral hepatitis prior to the development of portal hypertension.
Aim: To determine if small intestinal permeability is associated with hepatic fibrosis in non-cirrhotic patients with chronic viral hepatitis.
Methods: Subjects with chronic viral hepatitis without cirrhosis were prospectively recruited and compared to healthy volunteer controls. Small intestinal permeability was assessed by determining the ratio of plasma concentrations of lactulose and rhamnose (expressed as log L:R ratio), 90 minutes after oral ingestion of 5g lactulose and 1g rhamnose. Hepatic fibrosis was assessed by Transient Elastography (kPa) and hepatic steatosis by the Controlled Attenuated parameter (CAP). The limulus-amebocyte lysate assay was used to detect endotoxaemia in peripheral blood. Subjects were excluded if they had a liver stiffness measurement >11 kPa and platelet count $<150\times10^9$, drank alcohol within 24 hours of testing or had known gastrointestinal pathology. Statistical analysis was performed utilising SPSS.
Results: 39 subjects with chronic viral hepatitis (23 chronic hepatitis B, 16 with chronic hepatitis C) and 29 healthy volunteers completed evaluation. Small intestinal permeability was significantly higher in chronic viral hepatitis vs controls (2.78 ± 0.13 vs. 2.21 ± 0.18, $p=0.01$) (mean±SEM). In the chronic viral hepatitis group, there was a positive correlation between permeability and hepatic stiffness (Pearson's correlation $r=0.40$, p-value 0.01). There was no correlation between permeability and age ($p=0.46$), renal function ($p=0.26$) or CAP value (0.73), and no difference between those with and without the metabolic syndrome (p 0.61). The proportion of endotoxin-positive subjects was similar between chronic liver disease and healthy controls (2/39 vs 1/29, $p=1.0$).
Conclusions: Small intestinal permeability is increased in non-cirrhotic patients with chronic viral hepatitis and is associated with the degree of fibrosis. These data suggest that permeability is elevated prior to the development of portal hypertension.

P0542

THERAPEUTIC EFFECT AGAINST HEPATITIS B OF VARIOUS NUCLEIC ACID POLYMERS IN THE CHRONIC DHBV INFECTION MODEL

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Background and Aims: Nucleic acid polymers (NAPs) block the release of HBsAg from infected hepatocytes and can effectively clear the blood of HBsAg in human subjects with chronic HBV infection. When used in combination with immunotherapy, NAPs can achieve higher SVR rates in patients than when immunotherapy is used alone. The goal of this preclinical study was to examine the effect of various NAP modifications on their tolerability, liver accumulation and antiviral effect in vivo in the chronic DHBV infection model.
Methods: Three-day-old Pekin ducklings were infected with infectious duck serum (2×10^{11} VGE/ml of DHBV). NAP treatment was started in 14 day old animals via intraperitoneal injection with 10 mg/kg of NAPs 3 times / week for three weeks followed by autopsy analysis. All five NAPs used (REP 2055, REP 2139, REP 2163, REP 2165 and REP 2166) had the same sequence composition ([AC]20) but each comprised different modifications impacting the tolerability and stability of oligonucleotides. Tolerability was assessed by monitoring weight during treatment, injection site reactivity and findings at autopsy. NAP levels in the liver at the end of treatment were assessed by fluorescence-HPLC using a fluorescent PNA probe-based hybridization assay. Antiviral activity was assessed by monitoring serum DHBsAg and anti-DHBpreS antibodies by ELISA and serum and liver DHBV DNA by quantitative PCR.
Results: No significant changes in weight gain or injection site reactions were observed for any NAP. All NAPs tested reduced serum DHBsAg and this effect was more pronounced with REP 2055, REP 2139, REP 2163 and REP 2165. The most significant stimulation of anti-DHBpreSAg antibody response was observed in the REP 2139 and REP 2165 groups. Substantial reductions in serum DHBV DNA were found in all NAP groups. Drastic decrease in liver DHBV DNA was observed for four groups treated with REP 2055, REP 2139, REP 2163 and REP 2165. Importantly, liver accumulations for some NAPs that were less resistant to nuclease attack (REP 2055 and REP 2165) were 6–7 fold less at the end of treatment than for REP 2139 yet showed comparable antiviral activity.
Conclusions: The tolerability and liver accumulation of NAPs can be modulated significantly without affecting their overall ability to reduce serum DHBsAg and elicit other crucial antiviral responses in DHBV-carriers, suggesting important ways in which NAPs can be altered to minimize side effects and liver accumulation in human subjects receiving NAP therapy.

P0543

COMPARATIVE EVALUATION OF THE ARTUS HBV QS-RGQ ASSAY AND THE ROCHE COBAS AMPLIPREP/COBAS TAQMAN V.2 HBV TEST FOR THE QUANTIFICATION OF HBV DNA IN PLASMA

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Background and Aims: Hepatitis B Virus (HBV) infection is a major cause of chronic liver disease. Virological monitoring of HBV DNA is essential for the diagnosis, follow-up and treatment of infected patients. HBV viral load (VL) quantification on plasma samples is based on viral DNA extraction, followed by real-time PCR amplification. VL are standardized and expressed in International Units (IU)/mL. Numerous commercial kits are now available. Evaluation of a new assay should ideally be performed on diverse clinical populations.
The goal of this study was to evaluate the performances of the artus HBV QS-RGQ kit from QIAGEN.
Methods: We analyzed 230 samples from HBV-infected patients, and 12 negative samples (HBsAg negative patients). Patients were informed; plasma samples were taken for routine follow-up, and stored at −80°C until testing; VL were routinely quantified with Ampliprep/Cobas Taqman v.2 Assay (Roche), used as predicate device for method comparison analysis. Repeatability and reproducibility were evaluated using 3 VL levels of 2 samples (genotype A and D), analyzed 8 and 7 times respectively. DNA extraction was performed with the DSP Virus/Pathogen Midi Kit

on a QIASymphony SP/AS system, using 1 mL of plasma, and DNA elution in 60 μL buffer.
Results: Intra-assay and inter-assay coefficients of variation of the artus HBV QS-RGQ kit ranged from 0.4% to 2.8% and 0.3% to 8.1%, respectively. No negative sample was found positive. 52 samples were undetectable or below the lower limit of quantification, and 7 above the upper limit. 171 VL were within the linear quantification range for both techniques [1.5–7.3 log IU/mL]. Linear regression analysis showed an R2 value of 0.89. Bland-Altman analysis showed a good level of agreement with a median difference (Roche-QIAGEN) of +0.1 log IU/mL. 77% VL were within a −0.5/+0.5 log IU/mL difference range. A total of 20 samples were discordant between the two tests. The potential influence of gender, age, viral co-infections, ongoing treatments, as well as HBV genotype, on HBV quantification is currently evaluated.
Conclusions: In conclusion, the QIAGEN artus HBV QS-RGQ assay is specific, reproducible, and accurately quantifies HBV VL in chronically infected patients. Differences in technical protocols may explain the discrepancies observed. While the quantification range is a bit narrower than that of other available assays, these data suggest that the artus HBV QS-RGQ assay is suitable in monitoring HBV DNA levels according to current clinical practice guidelines.

P0544

INNATE AND ADAPTIVE IMMUNE RESPONSES CORRELATE WITH PEGINTERFERON ALFA TREATMENT EFFICACY IN CHRONIC HEPATITIS B PATIENTS (THE OSST IMMUNOLOGY STUDY)

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Background and Aims: The aim of this study was to characterize the immunological features responsible for improved treatment responses in 77 patients with chronic hepatitis B (CHB) treated with peginterferon (Peg IFN) alfa-2a after switching them from entecavir (ETV) therapy.
Methods: Peripheral natural killer (NK) cells, Toll-like receptors (TLRs), T cells, regulatory T cells (Tregs) and programmed death 1 (PD-1) were evaluated dynamically by flow cytometry. Response was defined as hepatitis B e antigen (HBeAg) seroconversion, hepatitis B surface antigen (HBsAg) loss, and HBsAg seroconversion (either as singular events or in combination at week 48)
Results: From week 12 to week 24, compared with ETV responders or Peg IFN alfa non-responders, Peg IFN alfa responders exhibited a significant decline in Treg proportions as well as a diminished negative regulation of $CD8^+$ T cells by Tregs. Those HBeAg-negative patients at baseline treated with Peg IFN alfa also showed significantly decreased Treg proportions and a higher rate of HBsAg seroconversion. Moreover, Peg IFN alfa responders showed a significantly higher increase in the $NKG2C^+$ NK cell proportions from baseline to week 12 and of $TLR2^+$ monocytes at week 12 than Peg IFN alfa non-responders.
Conclusions: Successful response to Peg IFN alfa correlates with an early significant restoration of impaired immune responses. Although antiviral treatment response can be achieved by both IFN and ETV, the underlying immunological features vary which may explain the generally observed difference in off-treatment durability of response between the two treatments, as well as effects on HBsAg.
negative regulation of $CD8^+$ T cells by Tregs. Those HBeAg-negative patients at baseline treated with Peg IFN alfa also showed significantly decreased Treg proportions and a higher rate of HBsAg seroconversion. Moreover, Peg IFN alfa responders showed a significantly higher increase in the $NKG2C^+$ NK cell proportions from baseline to week 12 and of $TLR2^+$ monocytes at week 12 than Peg IFN alfa non-responders.

P0545

TARGETING VIRAL DNA WITH CRISPR/Cas9 ROBUSTLY SUPPRESSES HEPATITIS B VIRUS

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Background and Aims: Hepatitis B virus is a 3.2kb DNA virus that infects the liver. Current anti-viral drugs efficiently suppress viral replication but do not clear viral episomal DNA (cccDNA). As a result, lifelong treatment is often needed to control viremia, underscoring the need for drugs or strategies that can effectively eliminate cccDNA and lead to a durable cure. The recent discovery of CRISPR (clustered regularly interspersed short palindromic repeat) as a bacterial adaptive immune system, and subsequent engineering of this system to precisely cleave DNA, provides a potential approach for direct targeting of HBV DNA in infected human hepatocytes.
Results: We first engineered a set of CRISPR/Cas9 guide RNAs targeting conserved regions in the HBV genome and screened for their utility *in-vitro* and *in-vivo*. Hepatoma cells were co-transfected with HBV expressing as well as CRISPR/Cas9 encoding plasmids and animals were hydrodynamically injected with those plasmids to analyze the effect on HBV gene expression and replication. We identified three guide RNAs highly potent for HBV inhibition that were further tested in HepG2215 cells, which stably express HBV from an integrated transgene and also maintain a stable pool of cccDNA. A kinetic analysis revealed a robust reduction in HBV replicative forms as well as in cccDNA levels over time. Analysis of CRISPR-mediated DNA cleavage revealed substantial cleavage presence in integrated HBV DNA but much less in residual cccDNA. This suggests that that cccDNA targeted by CRISPR/Cas9 may be rapidly degraded upon cleavage rather than repaired. Importantly, predicted off-target gene loci were not cleaved, further supporting the mechanism and specificity of this approach. Finally, hepatoma cells over expressing the HBV receptor NTCP and selected for the expression of HBV specific CRISPR/Cas9 guide RNAs were infected with HBV. A large attenuation of HBV infection was observed in CRISPR/Cas9 expressing cells, confirming the utility of this system in the context of natural infection, as well
Conclusions: The CRISPR/Cas9 system suppresses HBV replication and possibly eliminates cccDNA. Our study provides a proof of concept for targeting DNA viruses with CRISPR/Cas9 and highlights the possible utility of this approach as a curative anti-HBV therapy.

P0546

IN VIVO IMPACT OF PRECORE MUTATIONS OF HBV ON VIRAL REPLICATIVE CAPACITY, VIRAL PROTEIN PROCESSING, AND VIRION ASSEMBLY. MOLECULAR CORRELATES OF HISTOLOGIC AND IMMUNOHISTOCHEMICAL ASPECTS IN LIVER TISSUE FROM PATIENTS WITH CHRONIC HEPATITIS B

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Background and Aims: In chronic hepatitis B (CHB), ccc- and total intrahepatic DNA are the best markers of viral hepatic colonization and replicative capacity. Another interesting marker of viral colonization is histochemical staining of viral antigens. Such staining also reveals the intracellular localization pattern of viral antigens. In particular, HBsAg can be found either on intracytoplasmatic membranes or on the cell membrane in different patients. The molecular mechanisms determining this difference, and its pathogenetic or clinical implications are presently unknown
Methods: 35 consecutive untreated CHB patients have been enrolled for this study. In addition to classical virological and clinical parameters, intrahepatic cccDNA, total DNA (by real-time PCR) and intrahepatic viral antigen distribution (by immunohistochemical staining of HBcAg and HBsAg) were evaluated in bioptic liver tissue from these patients. Nucleotide sequencing of the pre-core and pre-s regions of viral genome was performed on intrahepatic and on peripheral blood virus
Results: A strong correlation between the frequency of HBcAg positive cells in the liver tissue and cccDNA molecules /10^5 cells was found. HBcAg positive cells also correlate with viral DNA load, HBsAg and especially HBeAg load in peripheral blood. By contrast, intrahepatic HBsAg positive cells did not significantly correlate with any of the different molecular parameters, but only with peripheral HBsAg. The different patterns of intracellular localization of HBsAg correlates significantly with viral replicative capacity (intrahepatic totalDNA/cccDNA). More insight into this aspect emerged by sequencing the pre-core region, which identified pre-core mutations (but not being HBeAg negative *per se*) as a strong correlate of intracytoplasmatic localization of HBsAg. In addition, a patient with grade 3 steatosis harbored a virus with a frameshifting deletion in the pre-core region which determined the expression of a 51 residues long peptide replacing HBeAg
Conclusions: This study indicates that pre-core mutations in the HBV genomes, in addition to abrogating HBeAg expression, lead to a significant reduction of viral replicative capacity, alter intracellular processing of viral proteins and virion assembly. Unexplained hepatic steatosis, a rare event in the natural history of CHB, might be a result of peculiar genetic determinants imposed by the immune system on the virus

P0547

PHYLOGEOGRAPHY OF GLOBAL HBV GENOTYPE D REVEALS THE CENTRAL ROLE OF CENTRAL ASIA IN DISSEMINATING THE EPIDEMIC

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Background and Aims: HBV genotype D shows a global expansion including Indigenous populations in Tibet and the Arctic. The aim of the current study was to estimate the pattern of dispersal of HBV genotype D using full-length genomic sequences.
Methods: We studied all full-length non-recombinant sequences of genotype D (N = 926) available on public databases. The migration events were inferred from viral phylogenies by character reconstruction using parsimony. This formed the basis of a metric that we used to determine whether a geographical unit (country or region according to WHO criteria) is actively spreading ("hub") or passively receiving ("sink") viral migrations.
Results: Based on combined statistical phylogeographic and additional analysis using the newly developed metric, we found that Turkey, Russia (mostly sampled from the C. Asian part of Russia), C. Asia, and L. America acted as hubs suggesting that they exported viral infections at a higher rate than the corresponding imported. On the other hand, India, Lebanon and New Zealand were sinks (higher rate for importing versus exporting viral migration) and Africa, E. Europe and Oceania/Pacific were classified both as sinks and hubs (no significant difference in importing versus exporting). For Greenland, S.E. Asia and Tunisia we found no significant migration, suggesting that they provide isolated areas ("islands") for viral dispersal. Finally for China, Belgium, Japan, W. Europe and Syria, we found no statistical evidence for their epidemic dispersal. Notably, all hubs apart from L. America exported within Eurasia, whereas for L. America we found significant dispersal on a global scale. We show similar patterns for C. Europe, whereas Oceania/Pacific exported to New Zealand and Africa to Tunisia and Europe.
Conclusions: We show that C. Asia and European areas have played a major role in disseminating genotype D epidemic within Eurasia. These patterns are probably due to expansion of Neolithic farmers from C. Asia westwards. On the other hand, exporting migration from L. America on a global scale was probably more recent than the C. Asian/European exports, given the recent introduction of genotype D in L. America as a result of the colonization of Americas by Europeans. Our study highlights the importance of westward patterns of viral dispersal mirroring the significant routes of human migrations during the Neolithic. One of the potential limitations is the non-uniform availability of sequences across different regions.

P0548

IMPAIRED ANTI-HBV VACCINE RESPONSE IN NON-CIRRHOTIC CHRONIC HCV PATIENTS COMPARED TO HEALTHY CONTROLS

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Background and Aims: Some immunogenicity studies of anti-HBV vaccine in patients with chronic HCV infection have demonstrated a diminished response ranging from 63.6% to 72.9% on seroconversion rate, compared to 90.9% to 93.9% in healthy controls. Aims: To evaluate the anti-HBV vaccination response in treatment naive chronic HCV patients without cirrhosis and compare them to healthy controls.
Methods: 110 chronic HCV adult patients without cirrhosis were randomized to receive anti-HBV vaccination regimen at standard 3 doses (0, 1 and 6 months) of 20ug, or higher dose of 40ug. Response to vaccination was measured by titers of anti-HBs 1 and 6 month after the last dose of anti-HBV vaccine. Healthy controls were negative to anti-HCV, anti-HBc, HBsAg and anti-HBs antibodies, and received standard 3 doses of 20ug at intervals of 0, 1 and 6 months, and were also evaluated for anti-HBs titers.
Results: Of the 110 HCV vaccinated patients, the seroconversion rate (anti-HBs ≥10 IU/mL) was 74.5% (82/110). Out of the 45 healthy controls vaccinated with standard dose, we observed a seroconversion rate of 93.3% (42/45).
Conclusions: Our study had demonstrated that chronic HCV patients without cirrhosis presented impaired anti-HBV vaccine

response compared to healthy controls, similar to data previously demonstrated in the literature. This impairment is apparently not overcome by exposure to double-dose anti-HBV vaccination regimen.

P0549

THE USEFULNESS OF CLIF-SOFA SCORE FOR PREDICTING SHORT-TIME MORTALITY OF PATIENTS WITH HBV-RELATED ACUTE-ON-CHRONIC LIVER FAILURE IN CHINA

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Background and Aims: Chronic liver failure (CLIF)-SOFA score was introduced to the diagnostic criteria for acute-chronic liver failure (ACLF) in recent by the European Association for the Study of the Liver (EASL)-CLIF Consortium. However, there were no relevant studies between CLIF-SOFA score and the patients who met Chinese diagnostic criteria for HBV-related ACLF. In this study, we investigated whether CLIF-SOFA score could predict 12-week mortality of patients with HBV-related ACLF in China.
Methods: The prospective study was conducted between 1 August 2013 and 1 August 2014. A total of 167 patients who met Chinese diagnostic criteria for HBV-related ACLF were recruited. At admission, CLIF-SOFA score was calculated according to the criteria from EASL-CLIF Consortium for each patient. All of the patients were followed up for at least 12 weeks. The predictive value of CLIF-SOFA score for short-term mortality was evaluated by receiver operating curve (ROC) curve. Kaplan–Meier survival curve and Cox's proportional hazards regression model were used to estimate survival rates and the risk factors for mortality.
Results: One hundred sixty-five cases completed the 12-week clinical follow-up. The overall mortality rate was 44.8%. According to the CLIF-SOFA score at admission, the patients were further divided into high score (CLIF-SOFA ≥10), medium score (CLIF-SOFA ≥8, but ≤9) and low score group (CLIF-SOFA <8). Kaplan–Meier survival curve showed that 12-week survival were significantly different among the three groups ($c2 = 18.460$, $P = 0.000$). The mortality rate showed a significant ($P < 0.05$) stepwise increase in the order of low score (30.5%), medium score (45.9%) and high score group (68.8%). The Cox proportional hazards regression model identified CLIF-SOFA score [$P = 0.006$, HR = 1.267, 95% CI (1.071–1.498)], MELD score [$P = 0.000$, HR = 1.107, 95% CI (1.049–1.169)], and total bilirubin [$P = 0.009$, HR = 1.044, 95% CI (1.011–1.078)] were independent factors significantly ($P < 0.05$) associated with survival. ROC curve showed that the predictive value of CLIF-SOFA was significantly higher than CTP ($P < 0.05$).
Conclusions: CLIF-SOFA score could distinguish the patients with HBV-related ACLF who had different outcomes. It might be used for predicting short-term mortality of patients who met Chinese criteria for HBV-related ACLF.

P0550

THE IMPACTS OF BASELINE CLINICAL CHARACTERISTICS AND HEPATITIS B VIRUS MUTATIONS ON CURATIVE EFFECTS OF CHRONIC HEPATITIS B TREATED WITH NOVAFERON

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Background and Aims: Novaferon, which is a new type of IFN, had approved by State Food and Drug Administration (SFDA) for clinical trials of new drugs on CHB therapy in 2009. To assess the impacts of baseline clinical characteristics of patients with chronic hepatitis B (CHB) and baseline hepatitis B virus (HBV) gene mutations on curative effects of CHB treated with Novaferon.
Methods: Enrolled patients accepted Novaferon monotherapy for 24 weeks and follow up for 12 weeks. Body mass index (BMI) of patients, genotype, basal core promoter (BCP) and precore (PC), reverse transcriptase (RT), S region variants of HBV were determined prior to Novaferon treatment. Aminotransferase (ALT), Hepatitis B e antigen (HBeAg), HBV DNA, Hepatitis B surface antigen (HBsAg) were determined at the beginning of treatment and 12 weeks after treatment. Then the present study evaluated the influence of age, gender, genotype, BMI, baseline ALT, HBeAg, HBV-DNA, HBsAg titer as well as BCP, PC, RT and S region variants on curative effects of chronic hepatitis B treated with Novaferon.
Results: Results In total of 126 CHB patients who finished the treatedment and follow-up, 38.9% obtained virological response and 32.5%, 25.4%, 44.4%, 23.8% obtained HBeAg clearence, HBeAg seroconversion, biochemical response and combined response respectively. The baseline DNA level of virological response group was significantly lower than no-virological response group; the baseline ALT level were significantly higher in HBeAg clearence group and HBeAg seroconversion group; female and lower BMI level was prone to acquired biochemical response. Stepwise logistic regression analysis showed that PC-P159T (ntC2288A), BCP-N118T (ntA1726C), BCP-L134L (ntA1775C/G/T) were independent influence factors for virological response. The frequencies of PC-G182C (ntG2357T) was significantly higher in groups with HBeAg clearance group and PC-S64A/T (ntT2003G/A), PC-W28STOP (ntG1896A) was significantly higher in HBeAg seroconversion groups, combined response respectively.
Conclusions: Conclusions Novaferon was an effective therapeutic drug for CHB patients, different response type was influenced by different clinical characteristics and mutations. Baseline mutations screening could predict curative effect of Novaferon efficiently and offered the optimal drugs for CHB patients.

P0551

THE CRISPR/Cas9 SYSTEM BEING AS A CUTTER FOR COVALENTLY CLOSED CIRCULAR DNA OF HEPATITIS B VIRUS

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Background and Aims: Current therapeutics block the production of new hepatitis B virus (HBV) DNA, but do not eliminate pre-existing viral reservoir of covalently closed circular DNA (cccDNA), thus curing less than 50% of patients.
Methods: We totally designed 20 CRISPR plasmids targeting at whole HBV genome, HBV DNA-integrated cell lines HepG2.2.15, HepAD38 and HepDES19 were tested as cell culture models. In HepDES19 cells, HBV e antigen was only produced from the enriched-HBV cccDNA but not integrated HBV DNA in chromosome. The system was also tested in HBV chronic infection mice, in which C57/BL6 mice was infected with PAAV8-HBV1.3 virus at least six months ago.
Results: When the cells were infected with HBV DNA-targeting CRISPR/Cas9 lenti viruses, HBV e antigen was decreased to ~91%, 84% and 51% in HepG2.2.15, HepAD38 and HepDES19, respectively. 18 of the 20 CRISPR/Cas9 systems had similar efficiency of inhibition except one was invalid and one was significantly higher efficiency. The inhibitory effect was significantly enhanced in combination of 3 cleavage sites. Sequence analysis confirmed that this CRISPR/Cas9 system efficiently cleaved and mutated HBV DNA target sites. CRISPR/Cas9 system exhibited a significant inhibition of production of viral proteins in HBV-infected mice as well
Conclusions: Although this system was able to remove internal viral genes from the host cell chromosome; it has higher efficiency to cleave extrachromosome HBV cccDNA. Our results suggest that the CRISPR/Cas9 system may be a useful tool for curing HBV infection.

P0552

VITAMIN D HAS NO DIRECT EFFECT ON HEPATITIS B VIRUS REPLICATION AND TRANSCRIPTION

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Background and Aims: Vitamin D is an important immune modulator that plays an emerging role in inflammatory and metabolic liver diseases, including infection with hepatitis C virus. In contrast, the relationship between vitamin D metabolism and hepatitis B virus (HBV) infection is less well characterized. Recently it has been demonstrated that low 25(OH)D3 serum levels are associated with high levels of HBV replication in patients with HBV (Farnik H, et al. [1]). Our aim was to determine in vitro the potential of vitamin D or its active metabolite calcitriol to directly affect HBV transcription and production.
Methods: HBV-transfected HepG2 cells (HepG2 215) and HBV-Luc construct (the luciferase ORF cloned downstream the HBV enhancer II and the core promoter) were used to assess the level of HBV transcription by determining the HBVRNAs level. In addition, the level of HBV replication was evaluated by determining the concentrations of HBsAg levels in the cell culture medium HepG2 215 (using specific enzyme-linked immunosorbent assay), the HBV-RNA by quantitative real-time PCR and by quantifying the level of cccDNA (using qRT-PCR on DNA extracted from HepG2 215 cells). The level of HBV transcription and replication were both assessed before and after treatment with vitamin D (0–200μM) or calcitriol (0–100 nM) for 48 and 72 hours.
Results: In HBV-transfected HepG2 cells, the administration of vitamin D or calcitriol did not suppress the secretion of HBsAg, the cccDNA expression, the replication of HBV and the HBVRNAs level. Increasing the dose of both vitamin D and clacitriol did not affect the findings.
Conclusions: Our results indicate that in HBV-transfected HepG2 cells there is no direct effect of either vitamin D or its active metabolite calcitriol on neither the transcription nor the replication of HBV. Vitamin D may be involved in HBV replication by other immune-mediated mechanisms.

Reference(s)

[1] Farnik H, et al. Hepatology 2013; 58(4): 1270–6.

P0553

POLO-LIKE-KINASE 1, A POSITIVE EFFECTOR IN HEPATITIS B VIRUS REPLICATION: THERAPEUTIC IMPLICATIONS

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Background and Aims: Polo-like-kinase 1 (PLK1), which plays pivotal roles in mitosis and is over-expressed in many human cancers, represents a promising druggable target in oncology. The X protein (HBx) of hepatitis B virus activates PLK1, which in turn induces degradation of chromatin regulating proteins SUZ12 and ZNF198, observed in human hepatocellular carcinoma. Whether PLK1 has a role in HBV replication remained to be determined, and represented the main objective of this study. Moreover we aimed at exploring if PLK1 inhibition could serve as a novel antiviral strategy against HBV.
Methods: Primary human hepatocytes (PHH) and differentiated HepaRG cells (dHepaRG) were primarily employed in this study as our HBV infection models, while initial studies employed HepAD38 cells. We generated and characterized HepaRG cells lines expressing in a tetracycline-dependent manner either individual HBV proteins or the constitutively active PLK1 mutant (i.e. T210D) for performing gain of function studies. Conversely, various commercially available Plk1 inhibitors and PLK1 siRNAs were utilized to inactivate PLK1, in loss of function studies, in order to determine the role of PLK1 in HBV replication.
Results: Firstly, we showed PLK1 activation in HBV infected cells, and that both HBx and HBc contribute to this activation. Gain- and loss-of-function studies for PLK1 demonstrated that PLK1 positively regulates HBV replication in differentiated hepatocytes. Next, antiviral properties of PLK1 inhibitors were investigated in HBV-infected hepatocytes. A 50% reduction (EC_{50}) in the accumulation of intracellular HBV DNA was quantified with a single administration of PLK1 inhibitor BI2536 at 5.0 nM. At this concentration, no effect on nuclear cccDNA, viral RNA synthesis, HBs/HBeAg secretion, was observed, indicating a post-transcriptional mechanism. BI2536 was not toxic in non-dividing cells up to 5000 nM. Interestingly, HBc accumulation was impaired in PLK1 inhibitor-treated cells, suggesting PLK1 targets HBc stability. In this respect, *in vitro* PLK1 kinase assays demonstrated that PLK1 phosphorylates HBc, although endogenous interaction between PLK1 and HBc was not detected in HepaRG cells. However, PLK1/HBc interaction was detected by co-IP in the HepAD38 model.
Conclusions: We have shown that HBV requires PLK1 activity to replicate efficiently in hepatocytes. PLK1 inhibition may therefore represent a novel antiviral therapeutic strategy for suppressing HBV replication.

P0554

HEPATITIS E VIRUS INFECTION INDUCES AN INNATE IMMUNE RESPONSE IN HUMAN CHIMERIC MICE

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Background and Aims: Hepatitis E Virus (HEV) is recognized as important pathogen in developing and industrialized countries leading to acute and chronic infections with considerable morbidity and mortality. Since our knowledge about the molecular biology and pathogenicity of HEV is still rudimental, we employed humanized uPA/SCID/beige (USB) mice to investigate viral host interactions.
Methods: Viremia and induction of human interferon stimulated genes (ISGs) and cytokines were determined by qRT-PCR and immunohistochemistry in humanized mice infected with HEV (genotype 1) and compared to uninfected chimeric animals.
Results: HEV viremia developed rapidly with up to 1×10^7 IU/ml within 4 weeks and remained stable for up to 35 weeks. HEV-infected human hepatocytes could be visualized by immunofluorescence using HEV orf2 specific antibodies and 1–5% of the human hepatocytes stained positive. Of note, although only a minority of the human hepatocytes stained HEV-positive, a clear induction of classical human ISG's (i.e. hMXA, hOAS, hISG15 and hSTAT1) was determined in the liver of HEV infected compared to uninfected mice. Pattern recognition factors hTLR2, hTLR3, hTLR4, hIFIH1 and hRig-I were also significantly upregulated (by factor 5–10 fold), indicating enhancement of innate recognition and defense mechanism in human hepatocytes. Furthermore, although human-specific cytokines like IFN-alpha, TNF-alpha and

IL6 were not or only slightly induced (i.e. IFN-beta and IL2; < factor 3), transcriptional levels of pro-inflammatory chemokines, such CXCL10 and CCl20, as well as IRAK2, appeared strongly enhanced (> factor 10) in HEV infected mice. Immunohistochemical staining of MXA and HLA confirmed that not only active HEV infected hepatocytes but also uninfected human hepatocytes could sense the infection.

Conclusions: Establishment of HEV infection provoked a clear enhancement of human innate defence mechanisms in chimeric mice, in the absence of adaptive immune responses. Elevated pre-treatment ISGs, pattern recognition factors and chemokines levels may contribute to liver damage and inflammation, providing a rationale for the severe clinical course of HEV genotype 1 associated liver disease.

P0555
INSERTIONS AND/OR DELETIONS IN THE MAIN REGULATORY REGION OF HEPATITIS B VIRUS SUGGEST MULTICODING OF THE X PROTEIN

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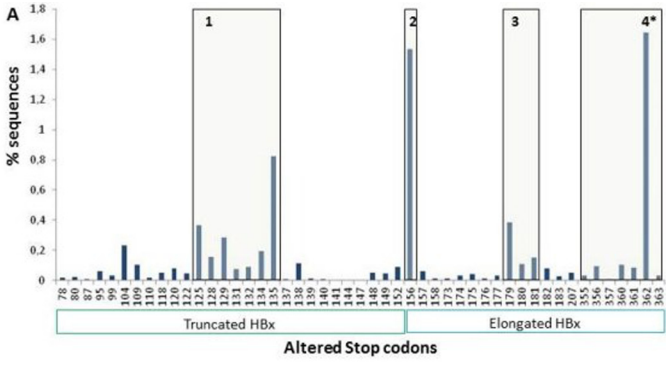
Background and Aims: The hepatitis B virus (HBV) main regulatory region, enhancer II (ENHII), encodes the preCore region and the C-terminal region of the multifunctional transactivating protein X (HBx). Nucleotide (nt) insertions and deletions (Ins-Del) in ENHII that modify HBx are associated with the severity of HBV infection.
Aim: Evaluate the presence of Ins-Del in ENHII and the possible effect of truncation or elongation of HBx on the HBV quasispecies.
Methods: Fifty samples from 50 antiviral-untreated patients with chronic active hepatitis were analyzed. HBV quasispecies from the nt 1596–1912 region was analyzed by ultra-deep pyrosequencing (454, Roche). Ins-Del were studied in the total of sequences (seqs) and variants (haplotypes).



B

	Ins-Del Variants	Nt localization	% Sequencies	% haplotypes
1	8-nt deletion	1754-1777 (TA2/TA3)	0.87	4.43
2	Duplication +/- deletion	1644-1770 (DDB1) 1754-1777	1.5	3.08
3	T insertion	1825	0.38	1.06
4*	T deletion	1825	2.91	4.32

DDB1, multifuntional cellular protein
* Stop codon stimated from the pattern sequence of each genotype

Results: A total of 960921 seqs, median 16734 seqs/patient (1905–57993) and 1039 haplotypes, median 17 haplotypes/patient (4–55) were analyzed. Overall, 128 different Ins-Del were detected in 7.1% of seqs and 27.5% of haplotypes. Ins-Del were observed in 47/50 samples (94%): median, 3.4% seqs with Ins-Del/patient (0–74.5%). Ins-Del changed the standard HBx stop codon (position 155), leading to 49 altered codons and resulting in a premature (truncated HBx) or late (elongated HBx) stop codon (Fig 1A). This occurred in 7.6% of seqs and 29.2% of haplotypes.
Main Ins-Del included (1) deletions between 1754–1777 causing elongated HBx, (2) duplications in 1644–1670 (with or without a deletion in 1754–1777) that modified the HBx interaction motif with the essential cell DNA damage-binding protein DDB1 and duplicated the target for C/EBP transcription factor, and (3) insertion or (4) deletion of a T in 1825, both encoding elongated HBx (Fig. 1B).
Conclusions: The significant presence of genomes that code for truncated or elongated HBx suggest a multicoding mechanism that could potentially affect the transcriptional activity of HBV ENHII and whose clinical relevance should be evaluated in further studies.

P0556
ANTIVIRAL EFFECTS OF NUCLEIC ACID POLYMERS ON HEPATITIS B VIRUS INFECTION

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Background and Aims: Hepatitis B virus (HBV) infection remains a major public health problem worldwide and current therapies are rarely able to cure HBV infection. Nucleic acid polymers (NAPs) have been shown to inhibit duck HBV infection *in vitro* and *in vivo* (Noordeen et al., 2013). NAPs have been shown to eliminate the hepatitis B surface antigen (HBsAg) from the blood (in ducks infected with the duck hepatitis B virus) but the mechanism whereby NAPs achieve the removal of surface antigen from the blood has yet to be clearly elucidated. Due to their phosphorothioate oligonucleotide structure, NAPs are chemically analogous to sulfated polyglycans such as heparin which are known to block entry of hepatitis B virus. In this study we investigated the *in vitro* antiviral activity of NAPs in HBV infected HepaRG cells and primary human hepatocytes.
Methods: NAP uptake into cells was assessed using Cy3 labeled NAPs. In order to evaluate potent effects of NAPs on HBV entry as well as post-entry, HBV infected differentiated HepaRG cells and primary human hepatocytes were treated with NAPs every two days starting at the time of infection or starting two days post-infection. The Elecsys HBsAg II quant automated system was used to quantitatively measure the secreted HBsAg. PreS1 containing particles were also assessed by ELISA and extracellular HBV DNA was measured by real-time PCR.
Results: Fluorescent NAPs were observed to enter into differentiated HepaRG cells and in primary cultures more particularly in hepatocytes rather than biliary cells. 5μM and 1μM NAPs significantly reduced extracellular HBsAg by 80% and 40% respectively as well as PreS1 containing particles when added at the time of infection. Interestingly, 5μM NAPs seems also to decrease extracellular HBsAg by 25% as well as PreS1 containing particles when added two days post-infection, suggesting NAPs exert a post-entry antiviral effect.
Conclusions: In this study, we showed a strong antiviral activity of nucleic acid polymers against HBV infection in HepaRG cells and primary human hepatocytes. Our results suggest that NAPs are able to block entry of HBV but also to have an effect on the replication cycle following entry of the virus which results in a reduction of HBsAg released into the supernatant. These antiviral activities both on virus entry and within the cells promise a strong potential of NAPs alone or in combination with already existing antiviral treatments.

P0557

EFFECT OF URSODEOXYCHOLIC ACID ON THE INTERACTION BETWEEN HEPATITIS B VIRUS AND SODIUM TAUROCHOLATE COTRANSPORTING PEPTIDE

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Background and Aims: Ursodeoxycholic acid (UDCA) had long been used for the management of chronic hepatitis B before potent oral nucleos(t)ide analog therapy was available. Recently, sodium taurocholate cotransporting peptide (NTCP) has been identified as a functional receptor of hepatitis B virus (HBV), and several *in vitro* studies reported that UDCA decreased expression of HBV in NTCP-expressing HepG2 cells, presumably via competitive inhibition of HBV-NTCP binding by UDCA. However, the effect of UDCA in HBV replication with respect to NTCP has not been fully known. In this study, the effect of UDCA on the interaction between HBV and NTCP was examined in non-neoplastic human liver cell line (PH5CH8). For *in vivo* study, the short-term effect of UDCA on the serum HBV DNA titers was examined in a retrospective cohort of chronic hepatitis B.
Methods: HBV was collected by ultracentrifugation of conditioned culture medium of HepAD38 cells. HBV precipitate was directly inoculated in the culture supernatant of PH5CH8 cells at various concentrations with or without UDCA NTCP mRNA and HBV rcDNA were quantified by real-time PCR. A cohort of 793 chronic hepatitis B patients without oral NA therapy was constructed from electronic medical record system of our hospital. Changes in HBV DNA levels after UDCA therapy were compared to control CHB group without UDCA therapy.
Results: Contrary to previous reports, UDCA pre-treatment increased intracellular HBV rcDNA in the direct inoculation model in a dose-dependent manner, whereas UDCA did not have significant effect on HBV replication when treated after HBV inoculation UDCA pre-treatment significantly up-regulated NTCP expression. UDCA did not affect serum HBV DNA levels in 42 CHB patients compared to 751 CHB patients without UDCA therapy.
Conclusions: UDCA up-regulated NTCP expression in non-neoplastic human liver cells with natural NTCP promoter, which might in turn induce internalization of HBV. Clinical use of UDCA did not affect serum HBV DNA levels in CHB patients, suggesting that UDCA has no significant effect on viral replication in CHB.

P0558

HBV INFECTION IS NOT AFFECTED BY THE INDUCTION OF FXR ACTIVITY BUT MAY LIMIT THE ACTIVITY OF FXR AGONISTS IN HUMANIZED MICE

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Background and Aims: The sodium taurocholate cotransporting polypeptide NTCP represents the main transporter for conjugated bile salts in hepatocytes and mediates HBV entry by serving as the binding site for the pre-S domain of the hepatitis B virus (HBV) envelope protein. Recently, HBV binding to NTCP was shown to limit its function, which in turn led to reduced nuclear translocation of the bile acid sensor farnesoid X receptor (FXR). Consequently, and possibly to maintain bile acid homeostasis, expression of genes involved in bile acid metabolism, such as CYP7A1 and SHP, were significantly altered in HBV-infected human hepatocytes in vivo (Oehler, Hepatology 2014). Since NTCP is also involved in drug uptake, HBV-mediated impairment of its function may limit response to certain drugs. *Aim* of this study was (I) to determine whether the induction of FXR activity mediated by the administration of FXR agonists may interfere with HBV replication and (II) to analyse their effectiveness on expression levels of genes involved in bile acid metabolism in HBV-infected human hepatocytes in vivo.
Methods: HBV stably infected (median viremia of 4.4×10^8 HBV-DNA/ml) and uninfected humanized uPA/SCID mice were treated for 3 weeks with PX20606 FXR agonist (0.2 mg/day, oral feeding), while vehicle fed infected and uninfected mice served as controls. Serological and intrahepatic viral loads, as well as human gene expression profiles, were determined by qRT-PCR.
Results: Despite modest enhancement of hSHP (0.2 log), induction of FGF19 (1.6 log) and reduction of hCYP7A1 (−0.9 log) transcriptional levels determined in HBV-infected mice after 3 weeks of PX20606 administration, no significant changes in HBV replication levels (both serum and intrahepatic) were observed. Remarkably, uninfected mice receiving the FXR agonist displayed more pronounced induction of hSHP (0.9 log), the transcriptional target of FXR and corepressor of hCYP7A1 transcription, stronger induction of hFGF19 (3 log), an inhibitor of hCYP7A1 transcription through FGFR4 activation, and a dramatic reduction of hCYP7A1 RNA levels (3 log), while FXR and NTCP expression levels remained comparable among all groups.
Conclusions: Although the gene expression changes induced by the FXR agonist on bile acid homeostasis had no significant impact on viral productivity, HBV infection appeared to limit the capacity of human hepatocytes to respond to FXR agonists that mostly mediate their effects through NTCP.

P0559

EVALUATION OF LARGE, MIDDLE AND SMALL HEPATITIS B SURFACE (HBS) PROTEINS IN HBeAg POSITIVE PATIENTS

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Background and Aims: HBsAg consists of three proteins called large (L-), middle (M-) and small (S-) HBs that differ in amino-terminal sequences and glycosylation status. Commercial HBsAg assays currently quantify the total amount of HBsAg. LHBs contains the preS1 domain which is necessary for entry and the preS-domains contain B and T cell epitopes, reflecting targets for specific host responses. So far there are no systematic data on HBs proteins but this may be relevant for the development of biomarkers, i.e. to predict treatment response to immune based therapies aiming at HBsAg loss.
Methods: Serum samples of 106 HBeAg positive patients, from two phase III/IV PegIFNα-2a trials were retrospectively analysed. Clinical and laboratory patient data was obtained from the completed trials and provided by Roche. We quantified the amount and the proportions of the three HBs proteins using a quantitative ELISA and well-defined monoclonal antibodies against the S-domain (total HBsAg), the preS1-domain (LHBs) and the N-glycosylated preS2-

domain (MHBs) of the HBs protein. SHBs was indirectly quantified by subtracting LHBs and MHBs from total HBsAg. The proportions were calculated in relation to total HBsAg.

Results: Baseline HBsAg levels measured with a commercial assay strongly correlated with our ELISA results for total HBs ($r = 0.97$, $p < 0.0001$), as well as the three individual HBs proteins. LHBs (median 1017 ng/ml) and MHBs levels (median 293 ng/ml) represent 8.3% and 2.8% of total HBs, respectively, whereas SHBs (median 10415 ng/ml) accounts for 88%. Interestingly, patients with genotype B showed significantly higher proportion of LHBs compared to genotype C (14% vs. 5.2%, $p < 0.0001$). Number of genotype A and D patients were insufficient for further genotype comparison.

Conclusions: The results validate an ELISA method for quantification of HBs proteins in sera from HBeAg positive patients with chronic hepatitis B. SHBs represents the commonest protein subtype, accounting for 88% of total HBsAg, with LHBs and MHBs accounting for 8% and 3%. The ratio of HBs proteins appears to be genotype specific: LHBs levels are significantly higher in patients with genotype B compared to genotype C. HBs subtypes represents a novel biomarker which deserves further investigation, particularly comparing genotypes and during administration of CHB treatments.

P0560

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISM rs2296651 IN SLC10A1 WITH HEPATOCELLULAR CARCINOMA AND LIVER CIRRHOSIS

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Background and Aims: Chronic hepatitis B virus (HBV) infection increases the risk for liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Sodium taurocholate co-transporting polypeptide (NTCP), encoded by *SLC10A1*, was recently identified as a receptor for hepatocyte entry of HBV. The SNP rs2296651 [A/G] has only been reported among Chinese populations, and the resulting NTCP p.S267F variant was shown to exhibit loss of bile acid uptake, and could neither bind to the HBV preS1 region, nor support HBV infection in cell cultures. Therefore, we aimed to assess the association of rs2296651 with HCC and LC.

Methods: The rs2296651 SNP was genotyped by the TaqMan platform in 3994 individuals with chronic HBV infection. Among 3994 individuals, 3470 were from the REVEAL-HBV study and 524 were patients with HBV-related HCC from the Taiwan Liver Cancer Network (TLCN). REVEAL-HBV is a community-based cohort recruited during 1991–1992 and followed-up every 6–12 months. The TLCN cohort is comprised of HCC patients from 5 major medical centers across Taiwan. Clinical and pathological information of patients were collected. DNA samples were obtained after approval by the research committee. The association of the SNP with HCC and LC was assessed by multivariate-adjusted odds ratios (OR_{adj}) with 95% confidence intervals (CI).

Results: The rs2296651 SNP was heterozygous (GA) in 678 (16.98%) and homozygous (AA) in 6 (0.15%) individuals with a minor allele frequency of 8.64%. The variant was observed in a subset of 100/781 (12.8%) HCC cases and 540/2945 (18.3%) non-HCC, non-cirrhotic controls ($P < 0.001$). After adjustment for age, gender, and serum ALT and HBV DNA levels, there was a significant association between the rs2296651 variant and HCC (OR_{adj} [95% CI] 0.57 [0.44–0.75], $P < 0.001$). In another subset, the variant was also lower in LC patients (86/607, 14.2%) than in healthy controls (559/3054, 18.3%) ($P = 0.01$). The variant was significantly associated with LC (OR_{adj} [95% CI] 0.64 [0.49–0.85], $P = 0.002$) after adjustment for other LC risk factors. There was no significant association between the rs2296651 variant and HBsAg seroclearance (OR_{adj} [95% CI] 0.88 [0.67–1.15], $P = 0.89$).

Conclusions: The rs2296651 SNP plays an important role in the development of LC and HCC. This finding is in accordance with previous reports on the function of p.S267F in HBV entry and infection, which is involved in the hepatocarcinogenic potential of the virus.

P0561

DIFFERENCES IN QUANTITATIVE COMPOSITION OF LARGE, MIDDLE AND SMALL HEPATITIS B VIRUS (HBV) SURFACE ANTIGEN (HBsAg) IN ACUTE AND CHRONIC HBV INFECTION

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Background and Aims: Quantification of HBsAg has been used as a marker to predict the course of HBV infections and response to antiviral treatment. The viral surface antigen consists of three components, large (L-), middle (M-) and small (S-) HBsAg, which differ in amino-terminal sequences and glycosylation status. In different ratios, all HBsAg components are part of infectious virions and non-infectious subviral particles. Quantitative analysis of HBsAg composition in serum can be a potential marker to assess the relative proportion of infectious virions. Additionally, there are evidences indicating that the L-HBsAg plays an important role in viral entry of hepatocytes. However, the clinical value of determining HBsAg components has not been established yet. In this study, we established a sensitive ELISA for quantitative detection of the different HBsAg components and determination of the HBsAg composition in patients with either acute or chronic HBV infections.

Methods: Microtiter plates were coated with specific antibodies and blocked with different reagents to optimize the test. L-, M- and S-HBsAg were quantified in serum samples of 23 patients (11 acute and 12 inactive chronic HBV infections) without antiviral therapy and a wide range of known HBsAg in total (mean 14.490 IU/ml, range 1.68–124,000 IU/ml) measured by a commercial assay (Abbott Architect). Three healthy patients served as negative control.

Results: Detection of lowest quantities (2–40 ng/ml) of all three HBsAg components was possible. The mean serum levels of HBsAg in patients with acute and chronic HBV infection were $4.81 \pm 4.82 \log_{10}$ ng/ml (range, $0.67 \log_{10}$ to $5.47 \log_{10}$ ng/ml) and $4.31 \pm 4.20 \log_{10}$ ng/ml (range, $1.63 \log_{10}$ to $4.76 \log_{10}$ ng/ml; $p = 0.128$). The mean ratios of L-, M- and S-HBsAg in patients with acute and chronic HBV infections were 25% versus 17% ($p = 0.073$), 8% versus 4% ($p = 0.016$) and 66% versus 78% ($p = 0.021$), respectively.

Conclusions: Different phases of HBV infection may be characterized by different ratios in HBsAg components. Quantitative analysis of HBsAg composition in serum can be a potential marker to characterize the course of HBV infections and the response to antiviral treatment.

P0562

MICRORNA-99 FAMILY MODULATES HEPATITIS B VIRUS REPLICATION BY TARGETING THE PHOSPHOINOSITIDE 3-KINASE/ Akt/mTOR SIGNALING PATHWAY

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Background and Aims: MicroRNA (miRNA) is a class of highly conserved small noncoding RNAs participating in regulation of various cellular processes like cell proliferation, differentiation, apoptosis, autophagy and viral replication. Evidently, miRNAs are able to modulate the host gene expression and thereby inhibit or enhance hepatitis B virus (HBV) replication. It was reported that the level of miRNA-99 family in peripheral blood corresponds with HBV DNA loads. Thus, we asked whether miR-99 family regulates HBV replication by targeting the specific cellular gene expression.
Methods: The level of miR-99 family members miR-99a, miR-99b, and miR-100 in primary human hepatocytes (PHHs) and hepatoma cells was determined by miScript RT-PCR. HepG2.2.15 cells were transfected with miR-99 family mimics and harvested at 4 days post transfection. HBV progeny, HBsAg and HBeAg from supernatant were separately determined by realtime PCR and chemiluminescence microparticle immunoassay (CMIA). The level of HBV replication intermediates (RI) from intracellular core particles was determined by Southern blot. HBV transcripts were detected by northern blot hybridization. And HBV promoter activity was also measured by using luciferase report vectors. Further, relative total or phosphorylated proteins were determined by western blot.
Results: Compared with primary hepatocytes, the level of miR-99 family members was down-regulated in hepatoma cells. Transfection of miR-99 family members resulted in a marked increase of HBV replication, progeny secretion, and antigen expression in HepG2.2.15 cells. To determine whether miR-99 family regulates HBV transcription or promoter activity, the results showed that transfection with miR-99 family members had no effect on them, suggesting that miR-99 family members regulate HBV replication through a posttranscriptional mechanism. Bioinformatic analysis and recent reports indicated that phosphoinositide 3-kinase (PI3K)/Akt/mTOR pathway was targeted by miR-99 family. Etopic expression of miR-99 family attenuated Akt/mTOR signaling pathway and could repress insulin-stimulated activation. Modulation of Akt/mTOR signaling by insulin or specific chemical inhibitors led to decrease or enhancement of HBV replication accordingly.
Conclusions: Our results thus demonstrate that miR-99 family promotes HBV replication through the PI3K/Akt/mTOR signaling pathway by regulation of a post-transcriptional process.

P0563

RNAi-BASED GENE THERAPY FOR CHRONIC HEPATITIS B: EVALUATION IN A MURINE MODEL

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Background and Aims: Current therapeutic options for chronic hepatitis B patients are unsatisfying, as nucleoside-analogs don't eliminate the virus and often need to be given life-long. A new therapeutic approach is to directly target viral mRNA transcripts for degradation by RNA interference. Long-term suppression of the viral transcripts can be achieved by a single dose of a vector encoding for a short-hairpin RNA (shRNA) and may allow restoration of antiviral immunity.
Methods: We designed recombinant Adeno-associated-virus (AAV) vectors to specifically address each of the proposed mechanisms by: (1) Co-expression of the shRNA with Ago2; (2) co-expression of the shRNA with a decoy RNA to neutralize the sense-strand; and (3) expression of the siRNA-sequence from a microRNA backbone using a liver specific polymerase III promoter. Vectors were injected i.v. into HBV-transgenic mice and compared head to head for efficacy of HBV-suppression and signs of toxicity.
Results: The conventional approach of a polymerase III promoter driven shRNA led to reduction of HBV-parameters by up to 97% but was accompanied by elevated ALT levels and loss of body mass. Co-expression of Ago2 with the shRNA from a single-stranded AAV reduced signs of toxicity with similar efficacy of HBV-suppression. Liver specific expression of the microRNA mimic showed ameliorated toxicity but was less efficient in suppressing HBV-replication. Co-expression of the sense-strand decoy with the shRNA not only prevented sense-strand off-target activity as revealed by transcriptome-analysis of liver RNA, and generally improved markers of toxicity. Most noteworthy, it also significantly enhanced HBV-suppression with long-term reduction by 99%.
Conclusions: We conclude that enhanced antiviral efficacy is due to the fact that the siRNA antisense-strand is more likely to be loaded into Ago2 in absence of the sense-strand. This underlies how understanding the mechanisms of RNAi-related toxicity and the rational design of new expression vectors can lead to the development of safe and efficient RNAi-based therapeutics for chronic hepatitis B.

P0564

INCREASED IL-17, TNF α SECRETION BY TFH AND THEIR SUBTYPE (TFH17) (CD4+CXCR5+CCR6+) CELLS HELP IN HBV SEROCONVERSION

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Background and Aims: The incidence of HBsAg spontaneous seroconversion is only 0.5%. Host immunity is mainly responsible for clearing the virus spontaneously. T follicular helper cells (TFH) are a special subpopulation of T helper cells that regulate B cell maturation and antibodies production through IL-21. On stimulation, TFH cells release pro-inflammatory cytokines, which help viral clearance. We investigated the role of TFH cells in HBV clearance, circulating CD4+ CXCR5+ (TFH) cells and their subsets; TFH1, TFH2 and TFH17 were studied.
Patients and Methods: *Inclusion criteria:* Patients with spontaneous seroconversion (group A, n = 11) were taken within 6 months of HBsAg loss and appearance of anti HBs >10 IU/ml (seroconversion) with negative HBV DNA. Patients in group B were treatment naïve, HBeAg+ve and persistently normal ALT (<40 wIU/ml) for >12 months. Serum HBsAg, Anti HBc (T), anti-HBs, quantitative HBV DNA, AST and ATL levels were determined for each patient in groups A (n = 11) and B (n = 20). Phenotypic expression of circulating TFH cells and their subtypes (TFH 1, 2 and 17) was analyzed by Flow cytometry. HBV specific response of TFH cells secreting TNF-α, IL-17A were determined by stimulating PBMC's with HBV surface pooled peptides (207–339) and HBV core pooled peptides (340–388) of HBV genotype D and PMA/Ionomycin. Data were analyzed using the non-parametric Mann–Whitney U test for comparing two groups.
Results: Spontaneously seroconversion of HBsAg was observed within 3–5 months of acute infection and patients showed anti-HBs titers in the range of 12 to 1000 mIU/ml. CD4+CXCR5+ (TFH) cells were significantly increased in group B compared to group A (43.3% vs. 34.7%, P = 0.01). There was HBV specific functional impairment of TFH1, TFH17 cells in group B compared

to group A patients. The peptide stimulation of TFH cells in group A compared to group B showed significantly increased frequencies of CD4+CXCR5+CCR6+TNFα+ and IL17+ cells producing pro-inflammatory cytokines, TNF-α (8.96% vs. 1.29%, p = 0.02) and IL-17A (15% vs 1.37%, p = 0.014).
Conclusions: Significantly increased IL-17A and TNF-α production by CD4+CXCR5+CCR6+ TFH17 cells may play a major role in HBV clearance and HBsAg seroconversion

P0565
CYTOKINE PATTERNS OF PATIENTS WITH HCV/HBV CO-INFECTION
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Background and Aims: In the natural course of HBV/HCV co-infection, different patterns of viral dominance have been documented based on HBV DNA and HCV RNA quantification. In the majority of cases, HCV seems to dominate and suppress HBV replication. The mechanisms are poorly understood but immune mechanisms may be one reason. It is known that HCV infection is associated with induction of ISG and high IP-10 levels. The aim of this study was to analyze immunological serum markers of HBV/HCV co-infected patients revealing an association with different dominance pattern.
Methods: In this cross-sectional retrospective study we analyzed 61 serum cytokines, chemokines and angiogenetic factors in 50 patients with respect to different serological patterns of HBV/HCV co-infection. Four pattern of viral dominance have been defined according to (Raimondo et al., 2006): (A) HBV dominance with HBV DNA >2,000 IU/mL and HCV RNA <600 IU/mL, (B) HCV dominance with HCV RNA >600 IU/mL and HBV DNA <2,000 IU/mL, (C) both viruses dominant with HBV DNA >2,000 IU/mL and HCV RNA >600 IU/mL, (D) no virus dominant with HBV DNA <2,000 IU/mL and HCV RNA <600 IU/mL. Univariate and Multivariate analyses were performed.
Results: Group A and B differ in concentration of FGFb, IFNg, VEGF, IL1a, IL2Ra, IL12p40, IL16, IL17a, IL25 p = *; MCSF, SCF, IP10 p = **; CTACK p = ***. All but one marker are higher in HBV dominant patients, while only IP10 is higher in HCV dominant patients (4,785 pg/mL vs. 1,820 pg/mL; p = **). Multivariate analyzes revealed that VEGF p = *, IP10 p = ** and CTACK p = *** differ independently. Comparing group A and C showed only a difference considering IP10 concentration, which was higher in group C (5,887 pg/mL vs. 1,820; p = *). Group B and C discern in concentration of IL1b, IL7, GCSF, PDGFbb, MIP1b, VEGF, IL1a, CTACK, MIF, TGFb2, TGFb3, IL17a, IL31 p = *; IL4, IL10, FGFb, IFNg, IL25 p = ** and IL12p70 p = ***, but not in IP10 concentration. Multivariate showed differences in IL1a and VEGF concentration p = *.
Conclusions: Host responses in form of cytokines, chemokines and anigogenetic factors show remarkable differences between different dominance pattern of HBV/HCV co-infection. For example, HCV dominance is associated with IP-10, which may help to explain the inhibitory effect on HBV. The pattern of immunological marker may also help to better understand the pathogenesis of HBV/HCV co-infection. Longitudinal analyzes are needed to evaluate the predictive value for endpoints like HBsAg loss, cirrhosis or HCC.

P0566
AUTOPHAGY OF CD4+T CELLS: A NEW MECHANISM OF SELF-REGULATION IN THE PROCESS OF CHRONIC HEPATITIS B VIRUS INFECTION
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Background and Aims: In the previous researches, we proved $CD4^+$T cells play important roles in regulating hepatitis B virus (HBV)-related inflammation and fibrosis. Recently, $CD4^+$T cells are found to achieve immune homeostasis via the autophagy. This study was designed to investigate the significance of $CD4^+$T autophagy in the process of chronic HBV infection.
Methods: Patients diagnosed as the inactive HBV carrier (n = 28), HBeAg-positive CHB (n = 48) and HBV-related cirrhosis (n = 55) were enrolled in this study. The autophagosomes of purified peripheral $CD4^+$T cells were observed under electron microscopy. The expressions of light chain 3 II (LC3II, specific marker for the autophagy) in $CD4^+$T cells were detected by immunofluorescence and flow cytometry. The autophagy associated signal proteins were tested by western blot. The *in vitro* stimulation experiments were also designed to determine the role of high mobility group box 1 (HMGB1) in the activity of $CD4^+$T autophagy and the associated downstream signal pathways.
Results: The mean number of autophagosomes in each $CD4^+$T cell was dramatically increased after chronic HBV infection compared to healthy subjects. The autophagy associated proteins in peripheral $CD4^+$T cells including LC3II, Atg5, Atg7 and Beclin-1 were all significantly upregulated when inflammation and fibrosis progressed after chronic HBV infection. Besides, the significantly positive correlations were found in HBeAg-positive CHB patients between serum HMGB1 levels and peripheral LC3II/CD4 ratios. Also, recombinant HMGB1 (rHMGB1) showed a strong ability to enhance the autophagy of $CD4^+$T from CHB patients *in vitro*, simultaneously with a dramatic up-regulation of RAGE expression on $CD4^+$T cells, rather than TLR2 or TLR4. Moreover, the intracellular phosphorylated JNK and ERK (not AKT or p38) proteins in $CD4^+$T cells were dramatically upregulated by rHMGB1 stimulation. Finally, the autophagy activity of $CD4^+$T cells could be significantly downregulated by either JNK inhibitor (SP600125) or ERK inhibitor (U0126).
Conclusions: The exacerbated autophagy of peripheral $CD4^+$T cells in the process of chronic HBV infection may prove to be an important mechanism of self-regulation which also indicates the inflammation degree.

P0567
GLOBAL PATTERNS OF HBV GENOTYPE D EPIDEMIC DISPERSAL
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Background and Aims: Genotype D is the only HBV genotype having a global expansion. This is reflected mainly by the subgenotypes D_1, D_2 and D_3 which are dominant in N. Africa, E. Europe and W. Asia. In contrast, D_4, D_5 and D_6 circulate mainly in indigenous populations in India, Oceania and Arctic. The aim of the current study was to estimate the levels of regional clustering for HBV subtype D in order to shed light on how the virus has been disseminated within different geographic areas over time.
Methods: We used 926 full-length non-recombinant genotype D sequences available on public databases. The number of sequences available for different regions (according to WHO criteria) were as follows: N. Africa and Middle East: 394, S. Asia: 157, W. Europe: 74, C. Asia: 44, Australasia: 40, Asia-Pacific: 36, E. Asia: 35, E.

Europe: 27, L. America: 25, Oceania: 23, Sub-Saharan Africa: 20, N. America: 16, C. Europe: 14, Caribbean: 11 and S.E. Asia: 10. Phylogeny reconstruction with bootstrap evaluation was conducted by the maximum likelihood method.
Results: The phylogenetic analysis suggests that HBV genotype D sequences form regional clusters at different percentages according to their geographic origin. Specifically 90% of the Australasian sequences form a single monophyletic cluster, followed by sequences from S.E. Asia (80%), Oceania (78%), Asia-Pacific (75%), Caribbean (64%), L. America (56%) and N. America (50%). Sequences from E. Asia (80%) and Sub-Saharan Africa (55%) form several monophyletic clusters while sequences from W. Europe (37%), S. Asia (26%), E. Europe (19%) and N. Africa and Middle East (16%) revealed the lowest monophyly levels. The C. Europe and C. Asia did not reveal any monophyletic pattern. Country-wise analyses showed the following monophyly patterns: Greenland: 100%, New Zealand: 97%, Japan: 75%, Tunisia: 66%, China: 61% and India: 27%. Absence of monophyly patterns was observed for Iran, Syria, Turkey, Belgium, Lebanon, and Russia (mostly sampled from the C. Asian part of Russia).
Conclusions: Our analysis suggests considerable differences in the patterns of HBV genotype D regional clustering around the globe. Notably for Eastern Mediterranean we found very low levels of regional clustering suggesting high levels of viral mobility. This pattern was distinct for Eastern Mediterranean in contrast to several areas in Africa, Asia and the Americas and it is indicative for the central role of this region for genotype D dissemination.

P0568

TGF-β SIGNALING IS ACTIVATED IN PATIENTS WITH CHRONIC HEPATITIS B AND IS REPRESSED BY SMAD 7 OVER EXPRESSION DURING LONG TERM ANTIVIRAL TREATMENT

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Background and Aims: Recently the contribution of TGF-β signaling in hepatic injury has been extensively studied. Although animal studies demonstrated that Smad7 induction ameliorates TGF-β/SMAD-mediated fibrogenesis, its role in human hepatic diseases is rather obscure. Our study was designed to explore the activation status of TGF-β/activin pathway in patients with chronic HBV hepatitis/cirrhosis (CHB) at diagnosis and the effect of successful antiviral treatment.
Methods: Liver biopsies from 37 HBeAg-negative CHB patients, (19 with active disease-CHB/d, 14 completely responders on 5 years antiviral treatment-CHB/r and 4 relapsers after treatment withdrawal-CHB/nr) were studied. Biopsies from 18 patients with chronic HCV hepatitis, 12 with non-alcoholic fatty liver disease (NAFLD), and three normals were also studied and served as controls. Liver mRNA levels of CTGF, all TGF-β isoforms and activins, their receptors and intracellular mediators (SMADs), were quantified using qRT-PCR and were compared and correlated with the intensity of liver inflammation and fibrosis staging. The expression and localization of pSMAD2 and pSMAD3 were also assessed by immunohistochemistry.
Results: CHB/d patients displayed a remarkable increased expression of *SMAD2* and *SMAD3* compared to CHB/r patients ($p < 0.001$). We observed an extensive nuclear positivity of pSMAD2 and pSMAD3 of the inflammatory infiltrates in both CHB/d and steatohepatitis patients, as well as variable nuclear positivity of the hepatocytes and biliary epithelial cells. Noteworthy, CHB/r patients displayed an approximately 3-fold increase of SMAD7 expression compared to CHB/d patients ($p < 0.001$). SMAD7 mRNA levels were significantly increased in patients with minimal inflammation, followed by a decrease as inflammation became severe. We observed that TGFB1, INHBB and SMAD4 mRNA levels are also significantly correlated with fibrosis grade, following a similar expression pattern ($p = 0.039$, $p = 0.006$ and $p = 0.008$, respectively). SMAD7 overexpression was also observed in NAFLD patients, despite activation of TGF-b/activin signaling, indicating that SMAD7 upregulation might effect in limiting the fibrotic process.
Conclusions: Molecular and immunohistochemistry data clearly demonstrate that TGF-β signalling is activated in CHB patients with active disease, while SMAD7 is up-regulated during the resolution of inflammation after successful antiviral treatment. SMAD7 overexpression might represent a mechanism limiting TGF-β-mediated fibrogenesis in CHB.

P0569

LIVER INFLAMMASOME EXPRESSION SIGNALING MAY BE ACTIVATED IN PATIENTS WITH CHRONIC HEPATITIS B AND REPRESSED DURING LONG TERM ANTIVIRAL TREATMENT

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Background and Aims: Inflammasomes are molecular platforms activated upon cellular infection or stress that trigger the maturation of pro-inflammatory cytokines such as interleukin-1b to engage innate immune defenses. The NLRP3 inflammasome is a cytosolic protein complex required for the development of sterile inflammation, that can further increase organ damage. Many liver diseases such as alcoholic steatohepatitis, non-alcoholic steatohepatitis, and drug-induced liver injury have sterile inflammation as a major component. Our study aimed to explore, for the first time, the liver NLRP3 inflammasome pathway activation status in patients with chronic hepatitis B (CHB) at diagnosis, and how it is affected during long term antiviral treatment.
Methods: Twenty HBeAg-negative CHB naive patients with active disease before treatment, 12 patients on complete virological remission after long-term 5 years antiviral treatment and 9 patients with chronic HCV hepatitis were enrolled in our preliminary study. Liver mRNA levels of NLRP3 (NACHT, LRR, and PYD domains-containing protein 3/cryoporin), IL (interleukin)1b, IL1Ra (interleukin-1 receptor antagonist), ICE (interleukin 1 converting enzyme or Caspase-1) and ASC (apoptosis-associated speck like CARD-domain containing protein), were quantified using qRT-PCR and correlated with histological liver inflammation and fibrosis. Cell-specific inflammasome expression in the liver by immuno-histochemistry was also performed.

Results: Molecular data suggest that liver NLRP3 inflammasome pathway signaling may be activated in CHB patients with active untreated disease: NLRP3 (p = 0.012), IL1Ra (p = 0.024) and ASC (p = 0.028) liver mRNA expression is significantly up-regulated in comparison with CHB patients on remission after antiviral treatment. No statistical difference was observed between active CHB and CHC, with only a trend of higher expression of IL1Ra (p = 0.082) in CHB.
Conclusions: Our preliminary study suggests for the first time that liver NLRP3 inflammasome pathway signaling may be activated in CHB patients with active untreated disease. This up-regulation could represent an additional mechanism of liver damage in CHB. Its repression may represent a candidate for novel therapeutic approaches.

Viral hepatitis: Hepatitis A, B, D, E – b. Clinical (except therapy)

P0570
OCCULT HEPATITIS B INFECTION IN COMPLETELY IMMUNIZED INDIVIDUALS NEGATIVE FOR ANTI-HEPATITIS B CORE ANTIBODY

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Background and Aims: Positive anti-hepatitis B virus (HBV) core antibody (anti-HBc) was considered a stigma of HBV infection. Here we challenged this dogma by investigating the prevalence of occult HBV infection (OBI) in anti-HBc-negative, complete vaccinees in Taiwan.
Methods: 705 participants of an epidemiology study for vaccine-preventable diseases in Taiwan had completed ≥3 doses of HBV vaccine in infancy and were negative for anti-HBc. Of them, 460 carried isolated anti-HBV surface antibody (anti-HBs) (HBsAg-negative, anti-HBs-positive, and anti-HBc-negative) and 245 tested negative for all 3 markers (triple seronegative). All samples were submitted for PCR detection targeted against HBV S and X/pre-C genes.
Results: Of the 460 samples carrying isolated anti-HBs, 26 (5.65%) were positive for two-target PCR detection (diagnosed as OBI). Of the 245 triple seronegative samples, 12 (4.90%) were positive. In the former group, prevalence of OBI significantly increased in individuals aged 6–10 years (P = 0.004), accompanied by a significant reduction of the anti-HBs titers (P < 0.001). Of the 38 OBI patients, 7 had HBV DNA >60 IU/mL (ranged from 280 to 18,820 IU/mL). Sequence analysis of the S gene revealed vaccine-escape like mutations with predominantly genotype A. Clinical importance of recognizing OBI in anti-HBc-negative vaccinees was illustrated by a case receiving stem cell transplantation.
Conclusions: OBI could occur in anti-HBc-negative, completely vaccinated individuals. In individuals carrying isolated anti-HBs, the highest prevalence of OBI was observed in 6–10 years of age, associated with age-dependent reduction of anti-HBs titer.

P0571
LIVER HISTOLOGY IN IMMUNE TOLERANCE PHASE PATIENTS WITH CHRONIC HEPATITIS B VIRUS INFECTION ACCORDING TO DIFFERENT ALANINE TRANSAMINASE LEVEL

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Background and Aims: A normal alanine transaminase (ALT) levels does not mean normal liver inflammation. We aimed to observe the difference of liver inflammation in immune tolerance phase chronic HBV infection according to two upper limits of normal ALT standards (30 U/L for males and 19 U/L for females vs. 40 U/L).
Methods: 202 patients were divided into low ALT group (≤30 U/L for males and ≤19 U/L for females) and high ALT group (31–40 U/L for males and 20–40 U/L for females). Ishak and Metavir system were used to evaluate liver inflammation and fibrosis.
Results: Significant difference of Ishak inflammation score and fibrosis score were seen between low ALT group and high ALT group. Positive correlation was seen between ALT levels and Ishak inflammation score or fibrosis staging score. The proportion of mild inflammation was 97.9% in low ALT group and 65.7% in high ALT group. Metavir fibrosis staging in all cases of low ALT group is F0 and F1. In high ALT group, the proportion of F0, F1, F2, F3, and F4 was 51.4%, 39.0%, 7.6%, and 1.9%, respectively. Different distribution of Metavir mild, moderate, and severe or fibrosis staging was seen in age and sex, but not in HBV DNA levels.
Conclusions: For immune tolerance phase patients, the lower ALT ULN is better than the current one in evaluating liver histology. Sex and age are associated with the degree of liver histology for ALT levels >30 in males or >19 in females.

P0572
THE IMPACT OF HEPATITIS B VIRUS (HBV) INFECTION ON PROGNOSIS OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AND THE OCCURRENCE OF HEPATITIS FLARES AFTER WITHDRAWAL OF PROPHYLACTIC ANTIVIRAL TREATMENT ON COMPLETION OF CHEMOTHERAPY

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Background and Aims: It is important to understand the impact of hepatitis B virus (HBV) infection on prognosis of patients with lymphoma receiving treatment. There is also no consensus on the duration of prophylactic antiviral treatment after cessation of chemotherapy. We analyzed the characteristics and clinical outcomes of diffuse large B-cell lymphoma (DLBCL) patients with HBV infection and compare with those without HBV infection. We also assessed the occurrence of hepatitis flares after withdrawal of prophylactic antiviral treatment on completion of chemotherapy.
Methods: Patient records were reviewed retrospectively from all patients aged 18 or above, with untreated DLBCL who had received chemotherapy between January 1996 and December 2010 in Tuen Mun Hospital, Hong Kong. The hepatitis B surface antigen (HBsAg) positive patients were given prophylactic lamivudine until 6 months after finishing chemotherapy. After chemotherapy and withdrawal of antiviral therapy, patients were followed up regularly to examine for hepatitis flare.
Results: 81 patients were recruited with 16 in the HBsAg positive group and 65 in the HBsAg negative group. The prevalence of hepatitis B among DLBCL patients was 20% in our cohort. The clinical characteristics were similar in both groups of patients. There was no statistically significant difference in overall survival between the two groups (p = 0.23). 4 of the 16 HBsAg positive patients (25%) had hepatitis after cessation of chemotherapy and prophylactic

lamivudine. One patient was given CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) therapy and the other three were given R-CHOP treatment previously. The onset of hepatitis ranged from 2 to 4 months (mean 3 months) after stopping lamivudine. These four patients had normal ALT levels before chemotherapy and stopped lamivudine 6 months after completion of chemotherapy. Lamivudine was resumed in these four patients and one patient required addition of adefovir because of the presence of the YMDD mutant. The liver enzymes gradually normalized in all these patients.

Conclusions: HBV infection did not appear to affect the clinical outcomes and prognosis of DLBCL patients given prophylactic antiviral treatment. A significant number of hepatitis flares occurred after 6 months of antiviral prophylaxis upon finishing chemotherapy. It is reasonable to consider prophylactic antiviral therapy to extend to at least one year on completion of chemotherapy.

P0573

A STRATEGY TO FAVOR THE ACCESS OF IRREGULAR AND REFUGEE MIGRANTS TO A SCREENING PROGRAM FOR HBV, HCV AND HIV INFECTION

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Background and Aims: In recent decades, Italy has become land of immigration from countries with intermediate or high HBV, HCV and HIV endemicity. Half immigrants are irregular or refugee frequently excluded from social life by linguistic, cultural and socioeconomic barriers also limiting their access to the healthcare services. The aim is to analyze the use of strategies favoring the access of irregular or refugee migrants to a screening program for HBV, HCV and HIV infection.

Methods: A screening to identify subjects with HBV, HCV or HIV infection was proposed to 926 migrants by a team (doctors, nurses and cultural mediators) skilled in managing problems of vulnerable groups. The screening, free of charge and with no bureaucracy, was accepted by 882 (95.2%) migrants, 625 irregular and 257 refugee, and performed at 4 territorial medical centers in Campania region, southern Italy. None of these 882 had been vaccinated against HBV.

Results: 78 (8.7%) migrants were HBsAg positive, 35 (3.7%) anti-HCV positive, 11 (1.3%) anti-HIV positive and 7 (0.8%) had a multiple infection. These 131 patients were addressed to Department of Infectious Diseases for further investigation and treatment. Of the 801 HBsAg negative patients, 373 (40.2%) were anti-HBc positive. All migrants with a detectable marker of HBV, HCV or HIV infection were unaware of their serological status. The HBsAg positivity rate was high (13.9%) in migrants from sub-Saharan Africa and intermediate in those from eastern Europe (6.1% of 194 cases), northern Africa (2.5% of 80 cases) and Indian–Pakistani area (3.2% of 126 cases). Anti-HCV was more frequent in migrants from eastern Europe (6.2%) and Indian–Pakistani areas (7%) than in those from sub-Saharan (3.8%) and northern Africa (2.5%). Anti-HIV was detected in 17 (3.8%) migrants from sub-Saharan Africa, in 5 (2.4%) from eastern-Europe, in 1 (0.8%) from Indian–Pakistani area and in none from northern Africa. Regarding single countries, the highest positivity rate was found in Burkina Faso subgroup for HBsAg (28.6%), in Ukraine and in Pakistani subgroups for anti-HCV (11.6%) and in Nigeria subgroup for anti-HIV (4.5%).

Conclusions: This investigation identified 131 migrants with HBV, HCV or HIV infection unaware of their serological status, suggesting the need of a strong action of Healthcare Authorities of Italy, may be using our model, to favor the access of migrant populations to healthcare services and to support screening and educational programs.

P0574

A SIGNATURE OF ELEVATED IMMUNE ACTIVATION IS OBSERVED IN THE PERIPHERAL BLOOD OF VIRALLY SUPPRESSED, HBeAg-NEGATIVE CHRONIC HBV PATIENTS

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Background and Aims: In the natural history of chronic HBV infection (CHB) loss of HBeAg and anti-HBeAg seroconversion precedes loss of HBsAg and anti-HBsAg seroconversion, the latter being considered the equivalent of achieving immunological control and functional cure. However, patients who achieve HBeAg-negative status with nucleos(t)ides (Nuc) therapy rarely achieve HBsAg loss and seroconversion. This suggests that host immunity to HBV might be compromised even with complete viral suppression under Nuc treatment. This study compares the immunological profiles in peripheral blood of healthy donors and virally suppressed CHB patients.

Methods: Whole blood, serum and peripheral blood mononuclear cells (PBMC) were isolated from healthy donors (HD) and virally suppressed, HBeAg-negative CHB patients (CHB). Transcriptional profiling of the whole blood was performed by RNA-sequencing (n = 6, HD; n = 8, CHB) and cytokine secretion profiles were assessed in PBMC cultures and directly ex vivo in matched serum samples (n = 12, HD; n = 8, CHB).

Analyte	IFN-α	IL-29	IL-12	TRAIL	MIP-1β
PBMC					
HD	3.6 (±5.5)	19.8 (±26.8)	0.59 (±0.96)	16.6 (±27.8)	7991 (±17682)
CHB	41.1 (±24.4)	149.6 (±69.3)	6.51 (±3.94)	139.7 (±48.0)	84982 (±31830)
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
Serum					
HD	4.6 (±6.2)	7.5 (±4.6)	0.33 (±0.22)	9.7 (±3.2)	216 (±82)
CHB	27.1 (±39.0)	15.6 (±4.0)	0.96 (±0.91)	23.9 (±9.1)	682 (±361)
P-value	0.06	<0.001	<0.05	<0.001	<0.001

Mean (±SD) shown for each analyte in supernatants of un-stimulated 24 h PBMC cultures (upper part), and directly ex vivo in serum (lower part); units are pg/ml. Statistical significance was calculated using an unpaired, 2-tailed T-test.

Results: Principal component analysis of the transcriptome data showed segregation of CHB versus HD. Gene set enrichment analysis identified an IL-12/T cell receptor-related signature that was induced in CHB but not HD. In vitro culture of isolated PBMC revealed significantly elevated secretion of several cytokines in CHB compared to HD, including type I and III IFN, and also IL-12. Of note, elevated levels of some but not all of those cytokines were also detected in corresponding CHB serum samples.

Conclusions: These data suggest that an activated immune signature is maintained in peripheral immune cells of virologically suppressed CHB patients. Continuous low level cytokine production may mediate enhanced feedback inhibition mechanisms in the signaling cascades of key anti-viral mediators, and hence contribute to overall suboptimal anti-HBV immunity.

P0575

LIVER FIBROSIS IN TREATMENT-NAÏVE HIV-INFECTED AND HIV/HBV-COINFECTED PATIENTS: ZAMBIA AND SWITZERLAND COMPARED

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Background and Aims: Differences in HBV transmission, environmental exposures and other hepatotoxic coinfections between sub-Saharan Africa and Europe may influence the development of HBV-related liver fibrosis. We compared the impact of HBV-coinfection on the prevalence of liver fibrosis in HIV cohorts in Zambia and Switzerland.

Methods: We included all participants with available measurements of HBsAg, ALT, AST and platelets at ART initiation in the Zambian IeDEA-SA hepatitis cohort and the Swiss HIV Cohort Study. Hepatitis C-coinfected patients were excluded. Liver fibrosis was evaluated using the AST to Platelet Ratio Index (APRI), with a ratio >1.5 defining significant fibrosis. Baseline characteristics were compared between HBsAg-positive and negative individuals in both cohorts with the Chi-square test. We used multivariable logistic regression to evaluate the association between HBsAg-positivity and significant liver fibrosis. The analyses were repeated using HBV DNA >20 IU/ml as the marker for HBV activity.

Table: Risk factors for significant liver fibrosis, by cohort

	Zambia (n = 689)		Switzerland (n = 2,057)	
	No. (%) with significant fibrosis	Multivariable analysis (95% CI)	No. (%) with significant fibrosis	Multivariable analysis (95% CI)
HBV-coinfection				
No	23/600 (3.8)	1	87/1962 (4.4)	1
Yes	9/89 (10.1)	2.96 (1.29–6.80)	14/95 (14.7)	3.68 (1.97–6.87)
Gender				
Female	7/358 (2.0)	1	6/466 (1.3)	1
Male	25/331 (7.6)	3.41 (1.40–8.34)	95/1591 (6.0)	5.18 (2.16–12.41)
Age, y				
16–40	20/503 (4.0)	1	45/1126 (4.0)	1
>40	12/186 (6.5)	1.65 (0.77–3.53)	56/931 (6.0)	1.38 (0.91–2.10)
African origin				
No	0	1	88/1727 (5.1)	1
Yes	32/689 (4.6)	1	13/330 (3.9)	1.38 (0.72–3.65)
High alcohol intake				
No	25/577 (4.3)	1	85/1875 (4.5)	1
Yes	7/112 (6.3)	0.97 (0.39–2.40)	16/182 (8.8)	1.60 (0.90–2.84)
Advanced HIV disease				
No	12/376 (3.2)	1	83/1834 (4.5)	1
Yes	20/298 (6.7)	1.70 (0.79–3.67)	18/223 (8.1)	1.58 (0.88–2.83)
CD4+ count, cells/μL				
≥200	14/378 (3.7)	1	61/1445 (4.2)	1
<200	18/311 (5.8)	1.32 (0.63–2.79)	40/612 (6.5)	1.39 (0.89–2.19)

Results: Among 689 ART-naive patients in Zambia (52.0% female, median age 34 years) and 2,057 in Switzerland (22.7% female, median age 39 years), 89 (12.9%) and 95 (4.6%) were HBsAg positive, respectively. Overall, 90.3% (131/145) of those with an available HBV viral load at ART initiation had detectable HBV DNA. Age, sex, alcohol consumption, and HIV disease markers were similar between HIV and HIV/HBV-coinfected groups in both countries. Median APRI score was 0.37 [95% confidence interval (CI) 0.26–0.57] in Zambia and 0.40 (0.30–0.60) in Switzerland and differences in APRI between HIV and HIV/HBV-coinfected individuals were similar across cohorts. In both countries, HBV-coinfected patients were more likely to have an elevated ALT (grade 1 or higher) at ART initiation (Zambia: 18% vs 9%, p = 0.01; Switzerland: 26% vs. 11%, p < 0.001). In adjusted analyses, HBV-coinfection and male sex were independent predictors of significant liver fibrosis in both countries (Table). The association between HBV-coinfection and significant liver fibrosis was similar when active HBV replication was used instead of HBsAg-positivity.

Conclusions: HBV-coinfected patients had approximately 3 times the odds of significant liver fibrosis at ART initiation compared to HBsAg-negative ones in Zambia and Switzerland. Despite the known differences in HBV natural history between these settings, the degree of liver fibrosis and the role played by other risk factors were similar.

P0576

IMPACT OF ORGAN AND NON-ORGAN-SPECIFIC AUTOANTIBODIES ON THE TREATMENT OUTCOME OF PATIENTS WITH HEPATITIS D VIRUS INFECTION

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Background and Aims: Chronic hepatitis delta virus (HDV) infection has been associated with the production of autoantibodies. However, their clinical significance has not been investigated. We reevaluated the production of organ and non-organ specific autoantibodies in a large cohort of patients with chronic HDV infection who participated in the Hep-Net/International Delta Hepatitis Intervention Trial (HIDIT-1), as well as their impact in treatment outcome.

Methods: We investigated the presence of antinuclear (ANA), antimitochodrial (AMA), anti-smooth muscle (SMA), anti-liver kidney microsomal (anti-LKM) and anti-parietal cell (PCA) antibodies by indirect immunofluorescence on HEp2 cells and rat liver-kidney-stomach sections and anti-LKM-3 antibodies by western blot using recombinant UGT 1.1, at baseline and at week 48 of treatment, in the sera of 87 patients with chronic HDV infection. Quantitative determination of HDV-RNA, HBsAg and HBV-DNA was performed by real-time PCR, the Architect HBsAg assay and Cobas TaqMan HBV test, respectively, at baseline, week 48 (end of treatment) and week 72.

Results: At baseline, ANA, SMA, AMA, anti-LKM, PCA and anti-LKM-3 were detected in 29/87 (33%), 24/87 (27%), 2/87 (2.3%), 3/87 (3.4%), 4/87 (4.6%) and 21/87 (24%) of patients, respectively. At least 1 autoantibody was detected in 56/87 (64.4%). At week 48, positive were 29/74 (39%), 20/74 (27%), 3/74 (4%), 5/74 (7%), 18/74 (24%) and 20/74 (27%) respectively, while at least 1 autoantibody was detected in 54/74 (73%) of the patients. ANA presence at baseline was associated with increased probability of HDVRNA negativity at week 72 (p = 0.04). The absence of SMA at baseline tended to be associated with lower HDVRNA levels at week 72 (p = 0.057) and amelioration of fibrosis in liver biopsy at week 48 compared to fibrosis at baseline (p = 0.05). The absence of PCA at week 48 was associated with lower HBsAg levels at weeks 48 (p = 0.01) and 72 (p = 0.01), biochemical response (p = 0.04) and amelioration of inflammation in liver biopsy at the end of treatment (p = 0.03). Finally, the presence of at least 1 autoantibody at baseline was

associated with increased probability of HDVRNA negativity at week 48.
Conclusions: We found increased incidence of anti-LKM-3 as well as of other autoantibodies in patients with chronic HDV infection. Although anti-LKM-3 antibodies do not seem to affect response to treatment, others may play a role in the virological, biochemical or histological response.

P0577
VACCINE EFFICACY OF COMBINATION VERSUS MONOVALENT HEPATITIS B VACCINES IN INFANTS AT HIGH RISK OF VERTICAL TRANSMISSION

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Background and Aims: There are concerns with regards to the efficacy of combination (DTaP-IPV-HiB-HBV) vaccine, especially in infants with high risk of vertical transmission, and there has been no study comparing the vaccine efficacy in those receiving three doses of monovalent HBV vaccine versus two doses monovalent plus one dose of combination vaccine in infants of chronic HBV-infected mothers. The aim of this study is to examine the anti-HBs antibody levels of both vaccine regimens in a cohort of these high-risk infants.
Methods: An observational cohort of term neonates was recruited from June 2009 to June 2014 from multiple primary to tertiary centres in Singapore. Anti-HBs antibody levels were measured at 9 months of age. Vaccine regimen administered were based on the individual institutions' practice.
Results: All term neonates received monovalent hepatitis B vaccine with hepatitis B immunoglobulin at birth with a repeat dose of monovalent hepatitis B vaccine at 1 month. They received either monovalent (n = 111) or combination vaccine (n = 95) at 6 months. Baseline maternal age (31.3 year vs 32.9 years), hepatitis B e-antigen positivity (24% vs 28%, p = 0.78) and birth characteristics, including gestational age (38 weeks versus 38weeks, p = 0.34), birth weight (3.13 kg vs 3.16 kg, p = 0.62) were similar between groups. The mean anti-HBs antibody were both not statistically different (478 IU/L vs 469 IU/L, p = 0.88). Vaccine failure rate (anti-HBs antibody <10 IU/L) was 4.5% vs 4.7% (p = 1.00). Vertical transmission rate (HBsAg or HBV DNA positive) was 1.8% vs 2.3% (p = 0.78).
Conclusions: For term neonates delivered by chronic HBV-infected mothers, both 3-dose monovalent and combination vaccine regimen showed similar vaccine efficacy, with no increased risk of vertical transmission.

P0578
HBsAg NEGATIVE ANTI-HBC POSITIVE SEROLOGY INCREASES THE RISK OF HCC IN CHRONIC HEPATITIS PATIENTS: A META-ANALYSIS

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Background and Aims: We performed a meta-analysis to ascertain at which extend patients with chronic hepatitis (CH) of different etiology and positivity for anti-HBc are at a higher risk to develop hepatocellular carcinoma (HCC) than those anti-HBc negative
Methods: Included in the meta-analysis were 26 studies meeting these criteria: investigating on the relationship between HBsAg-negative/anti-HBc-positive serology and occurrence of HCC, being a case–control or cohort study, providing relative risk or odds ratios and 95% confidence intervals, being available as full text written in English, being published and indexed up to September 2014.
Results: Twenty-six original studies met inclusion criteria, allowing a meta-analysis on 14,558 patients: the rate of HCC was higher in the 5,581 anti-HBc positive subjects than in the 8,977 negative (RR = 1.58; 95% CI: 1.49–1.69, $p < 0.0001$). The results were similar when groups of patients with a different stage of liver disease were singularly analyzed: RR = 1.30; 95% CI: 1.12–1.51, $p = 0.001$, in the 1,932 patients with cirrhosis and RR = 1.44; 95% CI: 1.18–1.87, $p < 0.0001$, in the 778 with chronic hepatitis. An independent association of anti-HBc positivity with HCC was similar both in the 16 studies carried out in Asia (RR = 1.62; 95% CI: 1.50–1.74, $p < 0.0001$) and the 10 studies in other countries (RR = 1.50; 95% CI: 1.35–1.70, $p < 0.0001$). Regarding the etiology, the relationship between anti-HBc positivity and HCC was investigated in HCV-related chronic liver diseases (RR = 1.52; 95% CI: 1.41–1.63, $p < 0.0001$). In 9 studies the data are presented according also to both anti-HBc- and anti-HBs-positivity. Compared to the 4198 anti-HBs/anti-HBc negative patients the rate of HCC was significantly higher in both the 935 anti-HBs/anti-HBc positive patients (RR = 1.17; 95% CI: 1.01–1.35, $p = 0.030$) and in the 719 anti-HBs negative/anti-HBc positive ones (RR = 1.75; 95% CI: 1.56–1.96, $p < 0.0001$). Besides, the 935 anti-HBs/anti-HBc positive subjects showed a risk of HCC significantly lower than those anti-HBs negative/anti-HBc positive (RR = 0.63; 95% CI: 0.55–0.72, $p < 0.0001$).
Conclusions: This meta-analysis show that in HBsAg negative CH patiens "isolated" anti-HBc is strongly associated with the presence of HCC, an association observed in all subgroups established by the stage of the disease, aetiology and ethnicity.

P0579
EPIDEMIOLOGICAL, CLINICAL AND VIROLOGICAL FEATURES OF HEPATITIS DELTA VIRUS INFECTION IN FRANCE

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Background and Aims: Hepatitis Delta Virus (HDV) infection often causes severe chronic liver disease. Epidemiology of HDV has changed in developed countries, because of sanitary improvement on one hand, and of population migrations on the other hand. Comprehensive data on HDV and on its societal burden are currently lacking in France.
Methods: HDV-infected patients were identified from the French National Reference Centre database. Epidemiological clinical and virological data, including HBV and HDV viral load and HDV genotyping, were collected on a standardized form from initial diagnosis to the last recorded follow-up.
Results: 1112 HDV/HBV co-infected patients were included in the study. The mean age was 37±10 years. Patients were predominantly male (69%) and migrants (86%), originating from sub-Saharan Africa

(53%), southern and eastern Europe (19%), Middle East (5%) Asia (7%), northern Africa (2%) and South America (0.3%).
16% were IV drug users, 19% were co-infected with HIV and 24% with HCV; 46% had a history of past or persistent excessive alcohol consumption, and 37% were overweight. 88% showed detectable HDV viral load (median value: 165000 copies/mL) and 47% detectable HBV viral load (median value: 400 IU/mL).
HDV-1 was the predominant genotype (76%) followed by genotypes typical of the sub-Saharan area: HDV-5 (18%), HDV-7 (3%), HDV-6 (2%) and HDV-8 (2%).
At first referral 28% of the patients showed histological cirrhosis, 15% one episode (or more) of hepatic decompensation and 2.7% hepatocellular carcinoma (HCC).
During the median follow-up of 4.4 years [1.4–8.9], 47% of the patients were treated for HDV, and 70% remained HDV-RNA positive; 20%, 10% and 6.5% of the patients developed cirrhosis, liver decompensation and HCC, respectively. Liver transplantation was performed in 14% of all initial patients and 5% died. Overall, the 10-year risk of cirrhosis, decompensation, HCC, liver transplantation, and death was 40%, 31%, 15%, 16% and 7%, respectively.
Conclusions: HDV infection in France is predominantly observed in migrants, mostly from sub-Saharan Africa. Genotype HDV-1 is largely predominant. Anti-viral treatment was effective in 30% of the cases. HDV is currently the most severe among the chronic viral hepatitis causes, being associated with very high risk of cirrhosis and related complications

P0580
GENETIC SIGNATURES SPECIFICALLY CLUSTERED IN IMMUNE ACTIVE HBsAg REGIONS CORRELATE WITH IMMUNOSUPPRESSION-DRIVEN HBV REACTIVATION: AN EXTENSIVE ANALYSIS OF HBV GENOME

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Background and Aims: To investigate genetic features along HBV genome underlying immunosuppression-driven HBV reactivation.
Methods: This study includes 127 patients (pts) (all genotype D): 47 with HBV reactivation (defined by Hwang, 2013), and 80 with chronic HBV infection, drug-naïve, as control. Genetic distance (using genotype D as reference and expressed as mean±SE) is used to estimate the extent of genetic variability in sequences of Pre-S1/S2 (aa: 1–163), HBsAg (aa: 1–226), RT (aa:1–344), Basal Core Promoter (BCP) (nt: 1–72), and PreCore/Core (aa: 1–112) obtained by population-sequencing. HBsAg ultra-deep sequencing (UDPS) is performed for 30 HBV-reactivated pts and 23 controls.
Results: The pre-reactivation HBV status is: 63.7% anti-HBc pos ± anti-HBs, 21.3% inactive carriers, 6.4% isolated anti-HBs, 4.3% active carriers, and 4.3% negative to all HBV markers. 53.2% of HBV-reactivated pts was treated with rituximab for hematologic malignancies, 14.9% with corticosteroids for chronic inflammatory diseases, 27.7% with other immunosuppressive chemotherapeutics. In 51.1% of pts, HBV reactivation occurs after completing immunosuppressive therapy (range: 1–48 months).
HBsAg genetic variability is significantly higher in HBV-reactivated pts than in controls (0.025±0.006 vs 0.018±0.005, $P < 0.001$). Such increase is not observed in pre-S1/S2, RT and BCP/PreCore/Core, suggesting that HBsAg variability gives a specific contribution of to HBV reactivation.
In particular, 78.7% of HBV-reactivated pts (vs 6.3% of controls, $P < 0.001$) carries mutations localized in immune-active HBsAg regions. Among them, 14 (M103I/T, L109I, T118K, P120A, Q129H/R, Y134H, S143L, D144E, G145A/R, S154P, E164D) reside in major hydrophilic loop (target of antibodies). Most of them are known to act as immune-escape. The others (C48G, V96A, L175S, G185E) reside in Class-I T-cell epitopes.
By UDPS, these mutations occur with an intra-patient prevalence >50% in 81.8% of HBV-reactivated pts indicating their fixation as predominant species. In controls carrying such mutations, their intra-patient prevalence ranges from 0.4% to 21.3% ($P = 0.003$).
Conclusions: HBV reactivation occurs in various clinical settings, often after completing immunosuppressive therapy, and correlates with a high genetic heterogeneity specifically clustered in immune active HBsAg regions. This supports the need to carefully monitor all pts at reactivation risk, and to assess the best time for the onset and duration of prophylaxis to prevent clinical complications.

P0581
PREDICTION OF HBsAg SEROCLEARANCE AND HBsAg SEROCONVERSION IN A COHORT OF INACTIVE EUROPEAN HEPATITIS B (HBV) CARRIERS: A PROSPECTIVE LONGITUDINAL STUDY (ALBATROS STUDY)

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Background and Aims: Single-point HBsAg and HBV DNA quantification at baseline were shown to be major predictors of spontaneous clearance of HBsAg during natural course of HBV infection in Asia.
The aim of the present study was to analyze dynamic viral and biochemical parameters within the first and second year of follow-up in order to predict HBsAg seroclearance and HBsAg seroconversion in a cohort of inactive HBsAg carriers from Europe.
Methods: 602 patients with HBeAg-negative HBV infection were prospectively followed. They were considered not to be candidates for antiviral therapy at study inclusion based on international guidelines. Biochemical, virological and non-invasive fibrosis parameters were performed at baseline (BL) as well as during annual follow-up. HBsAg, HBV DNA and biochemical values were compared between the following groups: patients with continuous positive HBsAg (group A), patients with HBsAg loss (group B) and patients with HBsAg seroconversion (group C) within 2 years of study entry.
Results: Data of 1, 2, 3 and 4 years follow-up were available in 288, 184, 101 and 52 patients. 10 patients lost HBsAg within the first 2 years of follow-up and 6 out of 10 achieved HBsAg seroconversion. Patients without HBsAg seroclearance (group A) had higher HBsAg titers at baseline (BL) compared to patients

with loss of HBsAg (group B) ($p < 0.001$) and patients with HBsAg seroconversion (group C) ($p = 0.002$). In group B+C patients log decay rate of HBsAg decline was 2.67 per year compared to 0.002 in the remaining patients (n = 174) without HBsAg loss ($p = 0.0002$). There was no significant difference of annual decline between group B+C patients. Levels of HBV DNA at BL were lower in group A than in group B and C patients ($p = 0.018$ A vs B; $p < 0.001$ A vs C) without difference of annual decay rate. AST values were significantly lower in group A vs C patients ($p = 0.038$). HDL cholesterin levels were higher in group A than group B and lowest in group C patients ($p = 0.038$). In contrast, ALT, GGT, LDL and total cholesterol did not show significant differences between the 3 patient groups during 2 years follow-up.

Conclusions: After 2 years prospective follow-up of 602 patients with low-replicative chronic hepatitis B spontaneous HBs antigen loss and seroconversion rates were low (5.4% and 3.3% respectively). In addition to HBsAg concentration at baseline the annual decay rate was a highly significant predictor for both HBsAg seroclearance and HBsAg seroconversion.

P0582

ADHERENCE TO GUIDELINES: HEPATITIS B SEROLOGY BEFORE STARTING IMMUNOSUPPRESSIVE THERAPY (RITUXIMAB, ANTI-TNF) IN AN URBAN AND RURAL REGION IN NORTH HOLLAND (NETHERLANDS)

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Background and Aims: The risk of reactivation of a chronic hepatitis B or an occult hepatitis B (OBI) during treatment with certain immunosuppressive medication is relevant with a significant morbidity and even mortality. Therefore national guidelines of different medical specialties advice to screen for hepatitis B virus prior to starting therapy. The aim of this study was to assess the adherence to these guidelines in patients with gastrointestinal, rheumatic and hematologic diseases in a urban and rural region in North Holland, a province in the Northwest of the Netherlands with a relatively low prevalence of hepatitis B.

Methods: A retrospective cohort study was conducted. 508 patients, who received rituximab, adalimumab, infliximad or etanercept (biologicals) in 2013 and 2014, were included using the record list of the hospital pharmacy. Many of these patients received their first biological earlier. The medical records and laboratory results were screened. When a patient was screened for hepatitis B one year prior to the start of treatment, this was accepted as screening according to the guidelines. Prior to the start of the study a survey was send to all medical specialist involved in the treatment with above mentioned substances to obtain information about the personal knowledge and application of the guidelines.

Results: 35% of all patients were screened for hepatitis B prior to starting a biological. A difference was found between specialty. The gastroenterologist tested in 68.9%, the hematologist-oncologist in 15.6% and the rheumatologist in 28.4% of the cases. We also looked at testing rate prior to the start of other immunosuppressant (azathioprine, 6-mercaptopurine, methotrexate en prednisolone), this was very low (3.9%).

There were no cases of reactivation in the whole study group.

When we look at testing, before a biological, through the years we see a significant positive trend from 2000 until 2014 with a steep increase of screening from 2009 (5% in 2009 and 67% in 2014, $P = 0.000$). These are the years were divers guidelines concerning this issue were published (2008–2014).

Conclusions: In conclusion, 35% of the patients were tested for hepatitis B before starting a biological. There was no case of reactivation in our low-prevalance region. With precaution we conclude that in a low prevalence area not following the guidelines is possibly without clinical consequences and can safe costs. Further research in high prevalence areas has to be done.

P0583

AN ANALYSIS OF RISK FACTORS OF SECONDARY INFECTION OF PATIENTS WITH HBV RELATED ACUTE-ON-CHRONIC LIVER FAILURE AND ITS IMPACT ON PROGNOSIS

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Background and Aims: Hepatitis B related acute-on-chronic liver failure (HBV-ACLF) has a poor prognosis with high mortality. Secondary infection is frequently occurred in these patients. The aim of this study is to evaluate its impact on clinical outcome and identify the potential risk factors for its development in these patients.

Methods: In this retrospective and case controlled analysis, ninety-eight patients with HBV-ACLF were enrolled and divided into two groups (infected 48 and non-infected n = 50). Clinical features and biochemical markers of these patients were collected (Table 1). Univariate analysis was performed by chi-square test using SPSS 19.0 software.

Results: The average time duration to occurance of secondary infection was (12.40±9.61) days after patients administration. Sites infections were occurred: abdominal infections (including spontaneous bacterial peritonitis) 13 cases (27.08%), respiratory infections 10 cases (20.83%), blood-borne infection 3 cases (6.25%), biliary tract infection 2 cases (4.17%), urinary tract infection 1 case (2.08%), skin infections 1 case (2.08%), location unknown 8 cases (16.67%), more than two and two parts of the infection 10 cases (20.8%). Patients survival in infected group were 25%, significantly lower than that in non-infected group (96%, $P < 0.001$). Univiarate analyses indicated that risk factors for secondary infection include age >45 years ($P = 0.046$), ascites ($P = 0.003$), hepatic encephalopathy ($P < 0.001$), hepatorenal syndrome ($P < 0.001$), serum total bilirubin >400 μmol/L ($P = 0.029$), serum alanine aminotransferase >410 U/L ($P = 0.029$), serum total protein <62 ($P = 0.027$), serum globulin <25 g/L ($P = 0.01$), platelet count $<100 \times 10^9$/L ($P = 0.026$).

Table 1. Baseline characteristics of patients with HBV-ACLF

	Overall (n = 98)	Infected (n = 48)	Non-infected (n = 50)	P value
Age (mean±SD)	40.83±12.21	43.19±13.85	38.88±9.84	0.046
Sex (M:F)	89:9	45:3	44:6	0.487
Liver cirrhosis	22 (22.45%)	8 (16.67%)	14 (28.00%)	0.228
Ascites	56 (57.14%)	31 (64.58%)	25 (50.00%)	0.159
HRS	10 (10.20%)	8 (16.67%)	2 (4%)	0.049
Bleeding esophageal varices	2 (2.04%)	1 (2.08%)	1 (2.00%)	1
HE	32 (32.65%)	24 (50.00%)	8 (16.00%)	<0.001
Albumin (g/L)	32.08±4.59	31.23±4.39	32.89±4.67	0.073
Albumin <35 g/L	76 (77.55%)	39 (81.25%)	37 (74.00%)	0.471
Globulin (g/L)	30.57±7.78	29.94±9.02	31.18±6.40	0.432
Total protein (g/L)	62.65±8.70	61.16±9.04	64.07±8.19	0.099
ALT (IU/L)	641.90±781.95	532.19±782.17	747.22±774.9	0.175
AST (IU/L)	473.30±563.55	446.35±632.87	499.16±493.07	0.645
Total bilirubin (μmol/L)	287.74±130.15	307.02±151.22	269.24±104.32	0.152
Serum sodium (mmol/L)	137.79±4.38	137.05±4.95	138.52±3.65	0.660
Prothrombin activity (%)	38.04±12.16	38.52±14.11	37.6±10.00	0.51
INR for prothrombin time	2.06±0.56	2.10±0. 70	1.99±0.38	0.216
Serum urea (mmol/L)	5.36±6.00	6.01±5.70	4.72±6.28	0.255
Serum creatinine (μmol/L)	71.11±41.49	78.16±50.82	64.21±28.55	0.072
Platelet count (10^3/μL)	107.71±40.81	100.89±35.26	114.25±44.89	0.105
WBC (10^3/μL)	6.63±2.21	7.07±2.34	6.21±2.12	0.052
Hemoglobin (g/dL)	124.94±21.37	120.40±21.33	131.34±20.01	0.174
MELD scores	21.27±5.90	22.36±5.79	20.1±5.95	0.062
CTP scores	10.21±1.67	11.16±1.42	9.26±1.32	0.878
HBV DNA (lg copies/mL)	5.77±1.71	5.97±1.64	5.56±1.77	0.099
HBeAg positive	11 (11.22%)	4 (8.33%)	7 (14.00%)	0.525

Conclusions: Secondary infection including SBP and pneumonia frequently occurred in patients with HBV-ACLF and significantly increased mortality. Early identification and intervention of the risk factors may improve the survival.

P0584

GENDER DIFFERENCES IN HEPATOCELLULAR CARCINOMA (HCC) SURVEILLANCE ADHERENCE PATTERNS IN PATIENTS WITH CHRONIC HEPATITIS B (CHB) IN A MULTICENTER ACADEMIC AND COMMUNITY COHORT STUDY

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Background and Aims: HCC surveillance is associated with improved survival of CHB patients. Although HCC risk is higher in males than females, it is not known if there are gender differences in HCC surveillance adherence. Our goal is to examine HCC surveillance adherence in males versus females and to determine surveillance adherence predictors.
Methods: We performed a retrospective cohort study of 1098 consecutive CHB patients without prior history of HCC and at least 12 months follow-up at a university medical center and 3 community gastroenterology and primary care sites in the U.S from 10/98–10/13. Patients were identified using ICD-9 diagnosis codes. Adherence to HCC screening by AASLD 2010 guidelines was categorized by imaging: optimal (every 6 months), suboptimal (6–12 months), poor (>12 months) and none.

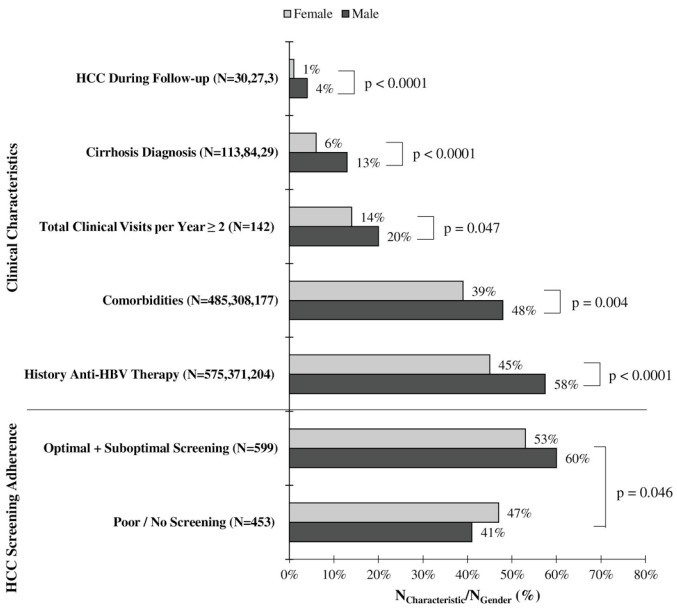

Figure: Clinical characteristics and screening adherence patterns of cohort by gender.

Results: The overall cohort was 51% male, 96% foreign-born, 97% medically insured, 92% Asian, and 61% from community practices. Males were slightly older (51.7 vs. 50.7, p = 0.17), had longer mean follow-up time (62.5 vs. 58.9, p = 0.11) but these differences were not statistically significant; however, and males were significantly more likely to receive anti-HBV treatment for CHB (57.5% vs. 45.0%, p < 0.001). Males had more severe progression of CHB such as cirrhosis diagnosis (13% vs. 6.4%, p < 0.0001) and HCC incidence (4.2% vs. 0.7%, p < 0.001) during follow-up compared to females. Males were also more likely to have two or more clinic visits per year (20% vs. 14%, p = 0.047) compared to females. According to AASLD HCC screening guidelines, males were more likely to undergo optimal or suboptimal screening (60% vs. 53%, p = 0.046) compared to females. On multivariate logistic regression also inclusive of age, sex, cirrhosis status, independent predictors for optimal screening adherence were anti-HBV therapy (OR = 2.3, p < 0.0001) and more frequent clinical visits per year (OR = 13.4, p < 0.0001).
Conclusions: In this large multicenter cohort study consisting of consecutive CHB patients followed at either academic or community practices, males were significantly more likely to have more severe progression of CHB, to receive antiviral treatment for CHB, to have more frequent clinic visits for CHB, and to have better HCC screening adherence relative to females. More frequent clinical visits may improve HCC screening adherence in both male and female patients with CHB, which ultimately can improve survival for HCC in patients of both genders.

P0585

DIFFERENCE OF SERUM CYTOKINES EXPRESSION BETWEEN HBeAg-POSITIVE AND HBeAg-NEGATIVE CHRONIC HEPATITIS B

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Background and Aims: The pattern of cytokines in sera was consistent with the level of inflammation during chronic hepatitis. By observing the difference of cytokines expression between HBeAg-positive and HBeAg-negative chronic hepatitis B (CHB), we aimed to explore the immune pathogenesis of HBeAg-negative CHB.
Methods: We investigated the expression of 30 cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-9, IL-10, IL-12p40, IL-12p70, IL-15, IL-17A, IL-17C, IL-21, IL-22, IL-23p19, IL-28A, IL-29, CCL5, CCL16, CCL20, CCL22, CXCL9, CXCL10, CXCL11, TNFRSF8, TNFRSF18, IL-6R, gp130, and TGF-β1) associated with HBV immune in sixty treatment-naïve CHB (30 with HBeAg-positive and 30 with HBeAg-negative) by a protein array. Cytokines expression among different ALT levels group and the correlation between cytokines and ALT levels were observed. Protein-protein interactions were analyzed by STRING.
Results: IFN-γ, IL-29, IL-7, IL-9, IL-15, and IL-6 with significant difference among different ALT groups in HBeAg-positive patients were not found significant difference among ALT groups in HBeAg-negative patients. IL-21 was the opposite. IFN-γ, TNFRSF18, IL-12p40, IL-17C, IL-1β, IL-12p70, IL-6, IL-15, and IL-10 with significant correlation in HBeAg-positive patients were not found significant correlation in HBeAg-negative patients. IL-21 and TGF-β1 were the opposite. Protein-protein interactions indicated that a close relationship between IFN-γ and STAT1, IL-6 up-regulating SOCS3, a close relationship between IL-21 and STAT3, IL-29 up-regulating Mx1.
Conclusions: Defection of IFN-γ, IL-29, and IL-6 expression and compensatory up-regulation of IL-21 is the characteristic of HBeAg-negative CHB and may be associated with the immune pathogenesis of HBeAg-negative CHB.

P0586

INFLUENCE OF HEPATITIS B e-ANTIGEN ON THE OUTCOME OF TWO NOSOCOMIAL OUTBREAKS OF HEPATITIS B IN NURSING HOMES IN GERMANY

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Background and Aims: Hepatitis B virus (HBV) causes acute or chronic hepatitis B. Outbreaks of HBV infections occur increasingly in skilled nursing homes in developed countries.
Methods: We investigated two recent outbreaks of hepatitis B in two nursing homes in Germany using serological tests, in addition to PCR, cloning and sequencing of associated HBV strains.
Results: The outbreak at location A included severe acute and fatal fulminant cases, while the outbreak at location B led mostly to asymptomatic or mild hepatitis B with very high viremia. Sequence analysis revealed at each location a different, but unique HBV strain that was able to induce high viremia of $>10^9$ virions/mL. At location B, a wildtype strain of HBV genotype A2 was found which is common in Central Europe. All viral isolates of the affected patients showed nearly 100% sequence identity and encoded HBV e protein (HBeAg) that serves as an immunemodulator during the incubation phase or chronic HBV infection. The severe acute and fulminant hepatitis B cases at site A were caused by a variant of HBV genotype D2 that was unable to express HBeAg, resulting in strong pathogenic immune reactions and high sequence variability. All patients had type 2 diabetes mellitus that is regarded as a risk factor for healthcare-associated transmission of HBV.
Conclusions: Transmission of HBeAg-negative HBV strains can lead to severe outcomes during local outbreaks of HBV. Improved hygiene, testing for HBsAg and active hepatitis B vaccination of residents in nursing homes, especially for persons with diagnosed diabetes should be taken into consideration to avoid the tragedy of hepatitis B transmission with fulminant outcome.

P0587

CAN HEPATITIS B SURFACE ANTIGEN LEVEL PREDICT SEVERITY OF LIVER DISEASE IN GENOTYPE E PATIENTS?

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Background and Aims: HBeAg negative chronic hepatitis B (CHB) infection is heterogeneous disease. CHB patients with persistently active hepatic necroinflammation (NI) and viraemia have higher rate of disease progression and complications related to liver disease. Surface antigen (HBsAg) level defined low replicative phase of CHB in genotype D patients. Higher HBsAg level predicts progression of liver disease and development of HCC in HBeAg negative CHB in genotype B and C patients. Correlation between HBV DNA and HBsAg level was noted in genotype D but not in genotype A patients. In this single centre cross-sectional observational study we aimed to evaluate whether HBsAg levels can predict severity of liver disease in genotype E HBeAg negative patients.
Patients: 259 HBeAg negative CHB genotype E patients underwent liver biopsy for assessment of liver disease (all HBV DNA >2000 IU/ml and/or abnormal ALT) between 2006–2014, median age 38 y, 61% males.
Methods: Demographic characteristics, viral and biochemical markers were evaluated near the time of liver biopsy. HBsAg levels (Abbott ARCHITECT®) were measured at same time-point. APRI and Kings (= age·AST·INR/Platelet) score were calculated. Patients were divided into 3 groups according to severity of fibrosis scoring on liver biopsy, Mild (F0–1), Moderate (F2–4), Severe (5–6) liver disease. The results are presented as median, ranges.
Results: The demographic, virological and biochemical parameters in 3 groups of patients according to severity of fibrosis are summarised in Table 1.
Patients were also divided into 2 groups based on their NI score, 0–3 and ≥4. There were significant differences in the ALT, HBV DNA, APRI, Kings Score and HBsAg/DNA ratio, but no difference in the HBsAg levels. Similarly in patients with APRI score (<1.5 and ≥1.5), HBV DNA was significantly higher in the group with APRI ≥1.5, but there was no difference in HBsAg levels. There was no correlation between the HBsAg and HBV DNA level in this cohort.

Table 1. The demographic, virological and biochemical parameters in 3 groups of patients according to severity of fibrosis

Parameter	Mild (n = 151)	Moderate (n = 84)	Severe (n = 24)
Age, years	38 (19–61)	39.2 (21–62)	41 (25–71)
Gender, male (%)	84 (55.6)	60 (71.4)	15 (62.5)
ALT, U/L (mean±SD)	40.7±76.8	69.2±136.9	130.3±187.7**
HBV DNA $\log_{10}$	3.51 (2.14–7.36)	3.63 (1.00–9.00)	5.59 (2.08–9.04)**
HBsAg level $\log_{10}$	3.90 (2.16–5.35)	3.80 (2.13–4.74)	3.53 (0.43–4.16)**
HBsAg/DNA ratio	1.06 (0.50–1.73)	1.00 (0.49–3.43)	0.60 (0.21–1.94)**
Knodell NI score	3 (0–6)	4 (1–9)	6 (3–9)**
APRI score	0.31 (0.13–12.78)	0.44 (0.14–7.53)	1.07 (0.19–2.68)**
King's score	4.71 (0.00–269.89)	6.84 (2.07–187.27)	18.82 (1.96–66.41)**

Comparison between 3 groups, *P < 0.05, **P < 0.001.
Kruskal–Wallis and chi-square test.

Conclusions: In our population, HBsAg level was lower in patients with more severe liver disease in CHB genotype E patients. Severe fibrosis was associated with higher HBV DNA and ALT level but lower HBsAg level and HBsAg/DNA ratio. There was no correlation between the HBsAg and HBV DNA level in this cohort. This probably highlights a differential HBsAg expression in genotype E patients with more advanced liver disease. The study population is small, hence further studies are required for confirmation of the above findings.

P0588

IMMUNE RESPONSE TO HEPATITIS B VACCINATION IN HIV INFECTED INDIVIDUALS WITH ISOLATED ANTIBODIES TO HBV CORE ANTIGEN

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Background and Aims: About 10% of HIV infected individuals are coinfected with HBV and about 15–45% have isolated antibodies to HBV core antigen (anti-HBc). Anti-HBc in absence of HBsAg may be encountered as a false positive result, as past infection with decrease of anti-HBs at undetectable levels or loss of anti-HBs. In this latter case the patients are not protected by HBV infection. We aimed to evaluate the anamnestic or primary response to standard HBV vaccination in HIV positive individuals with isolated anti-HBc.
Methods: Twenty-five HIV infected patients were included in this prospective study. They received a 3-doses HBV vaccination with recombinant HBV vaccine if they did not elicite an anamnestic response (anti-HBs >100 U/L) at week (w) 4 after the first dose of vaccine. Individuals with an anamnestic response were not vaccinated further. Clinical and laboratory data were evaluated at baseline, w 4, w 24, w 29.
Results: At baseline evaluation, the median CD4+ cells count was 562 (IQR 381–764); 13 patients were anti-HCV positive, 10 individuals had sexual risk factor for HIV infection and 15 were ex-IVDU. All patients were anti-HBe negative, had HIV-RNA

levels <50 copies/mL; 14/25 individuals were on ART including drugs active against HIV/HBV. Two of 25 patients (8%) elicited an anamnestic response. Eleven of 23 (48%) elicited a primary response. In total, the response to vaccine was obtained in 52% of individuals. Comparison of clinical characteristics showed that age, CD4+ cells count, transaminases, risk factors for HIV infection, and anti-HCV positivity were similar in the two groups. The responders were older than non-responders, a higher number of females females elicited humoral immune response; these data showing a trend towards significance P = 0.08 and P = 0.06, respectively. HBV-DNA measured at baseline by standard laboratory assay (real time-PCR) was negative in all individuals.

Conclusions: We found a low frequency of anamnestic response (8%) in this group of HIV infected patients, suggesting that the majority of individuals with isolated anti-HBc lost or did not form anti-HBs. This finding suggests also that testing for anti-HBc alone may not be the reliable assessment of protection from HBV infection.

P0589

IS HBV GENOTYPE PLAYING A ROLE IN PREDICTING POST-PREGNANCY ALT FLARE?

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Background and Aims: Pregnancy in chronic hepatitis B (CHB) patients represents a unique dynamic challenge for the host-immune interface. This cross-sectional, observational single-centre cohort study evaluated the importance of HBV genotypes on predicting post-pregnancy flares comparing virological (HBV DNA), serological (HBsAg levels) and immunological markers (plasma IP10 levels) markers during pregnancy (2nd trimester).

Patients: 312 CHB patients (median age 31.1years) were assessed at 2nd pregnancy trimester (1st visit median gestation week 26); 10 patients were treated with tenofovir (TDF) prior to pregnancy and 21 patients started TDF from gestation week 28 to minimise HBV transmission. 193 untreated patients (8% HBeAg+) were followed post-delivery (median 65days).

Methods: Plasma IP10, HBsAg, ALT and HBV DNA levels were measured by ELISA [pg/ml], Abbott ARCHITECT® assay [log10 IU/ml], AutoAnalyser [IU/l] and real-time TaqMan PCR [log10 IU/ml] respectively at 2nd pregnancy trimester and 1st post-pregnancy visit. The results were compared in 193 untreated patients based on ALT levels post-pregnancy: hepatic flares (ALT >40 IU/l and two-fold pregnancy level) (HF) (n = 46) vs. normal ALT post-delivery (n = 147). HBV genotypes were evaluated by direct sequencing in patients with HBV DNA >50 IU/ml. Fibroscan LSM was measured in all patients post-pregnancy (median 3.9 kPa).

Results: Patients with HF (24%) were more likely HBeAg+ (OR 11.2), had high IP10 (>200 pg/ml) (OR 9.3), HBV DNA (>10000 IU/ml) (OR 2.4) and low HBsAg/HBV DNA ratio (<0.85) (OR 6.2) at 2nd pregnancy trimester. The HBV genotypes distribution was following: A 10%, B 6%, C 7%, D 10%, E 41% and not typeable (NT) due to low HBV DNA in 26% patients. Post-pregnancy flares were more frequent in patients infected with genotypes B (40%), C (47%) and D (47%) than genotypes A (27%), E (20%) and NT (12%) ($p < 0.05$). When individual parameters were assessed only high pregnancy IP10 levels were significantly higher across all genotypes in patients with HF in comparison to normal ALT patients (median: 234 vs. 143, $p < 0.05$). Only in genotype B patients higher pregnancy HBV DNA (median: 4.93 vs. 2.87, $p = 0.01$) and HBsAg (median: 4.1 vs. 2.96, $p = 0.01$) were predictive of ALT flares.

Conclusions: Post-pregnancy flares were more frequent in genotypes B, C and D chronic hepatitis B patients and high pregnancy IP10 level was predictive of flares across genotypes.

P0590

MODIFIED MODEL FOR END STAGE LIVER DISEASE SCORE ACCURATELY PREDICTS DEATH OR LIVER TRANSPLANTATION IN ACUTE FLARES OF CHRONIC HEPATITIS B

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Background and Aims: For patients chronically infected with hepatitis B virus, acute flares of chronic hepatitis B (AFOCHB) can be severe and fatal. The decision on whether to transplant or not is often a difficult one. The current study aims to determine the predictive value of short-term outcomes using the MELD score and its modified counterpart in AFOCHB patients.

Methods: Patients admitted with AFOCHB with ALT >5× upper limit of normal and HBV DNA of at least 4 logs IU/mL were included. Laboratory data was collected at the time of admission, at day 7, and at day 14. The MELD-ALT-Platelet (MAP) score was derived by additional points to the MELD score according to the ALT level (+1, +2, and +4 points for ALT ≤2000, 2000–3000, and >3000 respectively) and platelet level (+1, +2, +3, and +7 points for platelets ≥150, 100 to <150, 50 to <100, and <50 respectively).

Results: A total of 210 patients with AFOCHB were included. The outcome measured was either death or liver transplantation. Within 30 days, 25 (11.9%) patients died and 91 (43.3%) underwent liver transplantation. The AUROC of INR, MELD, and the MAP score at the time of admission to predict day 7 outcome was 0.935, 0.945, and 0.950 respectively. At day 7, 179 patients survived without the need for liver transplantation. The AUROC of INR, MELD, and MAP score at day 7 to predict day 14 outcomes were 0.869, 0.867, and 0.871 respectively. By day 14, 159 patients remained alive without the need for liver transplantation. The AUROC at day 14 for INR, MELD and MAP to predict day 30 outcomes was 0.834, 0.878, and 0.912 respectively. Although a high AUROC was observed for day 0, 7, and 14 MAP to predict day 7, 14, and 30 events respectively, the accuracy was reduced when day 0 MAP was used to predict events at day 14 and 30, with AUROC of 0.819 and 0.764 respectively. A similar trend was observed when day 7 MAP was used to predict day 30 events, achieving an AUROC of 0.772 (compared to 0.871 for day 14 events). A MAP score of <25 at day 0, day 7, and at day 14 was associated with no events at day 7, 14, and 30 respectively. For those presenting with MAP ≥35, the rate of events by day 7, 14 and 30 was 72%, 88%, and 96% respectively.

Conclusions: Severe AFOCHB can be associated with high rates of mortality and LT. The MAP score at different time points can accurately predict short-term outcome to provide useful prognosis and determine the urgency of LT workup.

P0591

HBV VACCINATION STATUS IN PATIENTS WITH END STAGE PULMONARY DISEASE EVALUATED FOR LUNG TRANSPLANTATION

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Background and Aims: In times of limited donor resources, anti-HBc positive organs can be accepted for lung transplantation in order to increase the number of donors. Transplant recipients should be vaccinated against hepatitis B during transplant evaluation to prevent HBV infection. However, response after HBV vaccination has not been evaluated in patients with end stage pulmonary disease.

Methods: Anti-HBs titers of anti-HBc negative patients with end stage pulmonary disease evaluated for lung transplantation

were analyzed with the Architect® system (Abbott, Germany). Responders, partial responders, or non-responders after HBV vaccination were defined by anti-HBs titres >100 IU/L, 10–100 IU/L, and <10 IU/L, respectively.
Results: 28 patients were analyzed (46% female, age 57±8 years, COPD n = 18/28, long-term prednisolone therapy n = 5/28, history of smoking n = 24/28) and vaccinated at baseline either with Engerix® (n = 11), Twinrix® (n = 11), or HBVAXpro® (n = 1). Three and more or two vaccinations were performed in 14 (50%) and nine (32%) of cases. Five individuals (18%) were not vaccinated. Response, partial response, and non-response after vaccination were observed in 5/23 (22%), 7/23 (30%), and 11/23 (48%) of patients, respectively. Individuals with three or more vaccinations did not develop anti-HBs titres >100 IU/L more frequently than patients with two vaccinations (n = 4/14 vs. n = 1/9). Response to vaccination did not correlate with gender, pulmonary disease (COPD vs. non-COPD), long-term prednisolone therapy, or smoking status.
Conclusions: Only 5/28 (18%) of patients evaluated for lung transplantation showed anti-HBs titres >100 IU/L. Thus, serological control of anti-HBs titres after vaccination should be mandatory prior to transplantation. In case of transplantation of an anti-HBc positive donor organ, the majority of recipients needs therapy with a nucleos(t)ide analogue to prevent HBV reactivation after surgery.

P0592
A CROSS-SECTIONAL STUDY ON INTRAHEPATIC CHOLESTASIS INDICATORS OF VIRAL HEPATITIS PATIENTS

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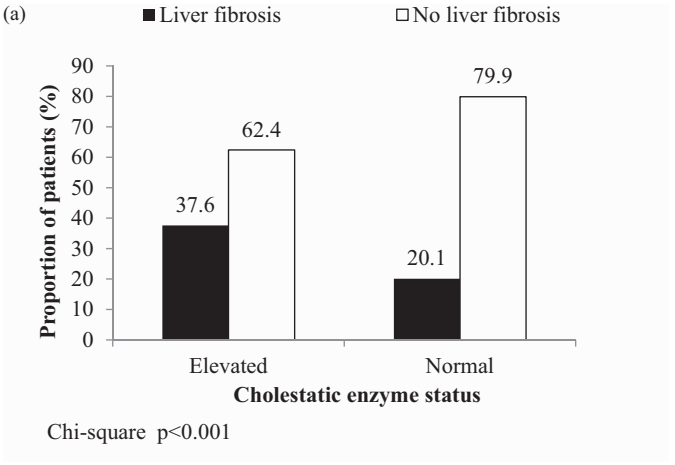
Background and Aims: Intrahepatic cholestasis (IHC) is a hepatocellular or cholangiocellular disorder which can be caused by viral hepatitis. The cut-off levels of serum alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) in diagnosing IHC are still debated. Although the patients are clinically asymptomatic, ALP or GGT can or remain abnormal. This study is to investigate the abnormality of IHC indicators (ALP, GGT) for in-patients with viral hepatitis when they are being discharged, and to explore the correlation between IHC indicators and liver fibrosis.
Methods: It is a multi-center, cross-sectional study. A total of 1000 hospitalized patients with viral hepatitis were recruited from five big hospitals. Demographic characteristics, clinical and laboratory data including IHC indicators and liver fibrosis indicators (hyaluronic acid and type IV collagen) were collected. Chi-square and multivariate logistic regression were performed to determine the correlation between abnormal IHC indicators and liver fibrosis.
Results: 998 of 1000 patients were included in the analysis (Table 1). 560 patients (56.17%) had abnormal IHC indicators at discharge. Comparing to patients with normal IHC indicators, patients with abnormal IHC indicators had significantly more abnormal liver fibrosis indicators (hyaluronic acid and type IV collagen; severer Child–Pugh Classification. both $p < 0.001$). Multivariate analysis showed that patients with abnormal IHC indicators had significantly higher risk to have abnormal liver fibrosis indicators ($p = 0.0236$, OR = 1.542), and higher trend to have higher Child–Pugh Classification ($p > 0.05$, OR = 1.238).
Conclusions: More than half of the patients with viral hepatitis had abnormal IHC indicators at discharge, which are correlated with liver fibrosis in clinical practice. Therefore, monitoring and following up on IHC indicators after discharge are recommended for viral hepatitis patients.



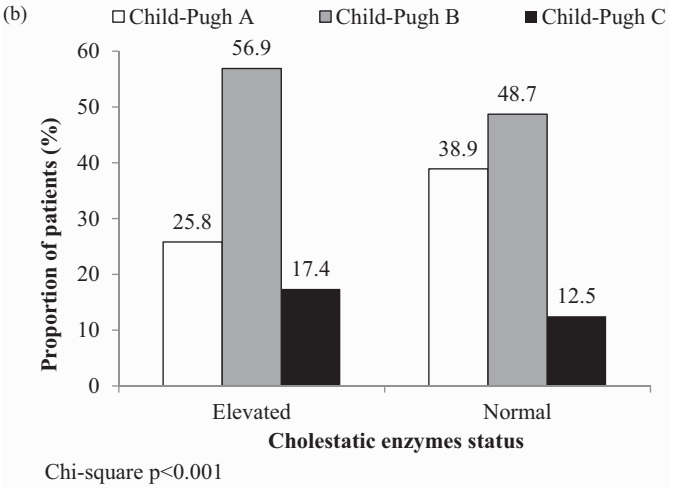


Figure 1. Distribution of (a) liver fibrosis and (b) Child–Pugh Classification according to abnormal IHC indicator status.

Table 1. Multivariate analysis on the correlation between abnormal IHC indicators and liver fibrosis or Child–Pugh Classification B and C

Indicators	ALP or GGT (OR)	p-value
Abnormal hyaluronic acid and Type IV collagen	1.542	0.0236
Child–Pugh Classification B and C	1.238	>0.05

P0593
PREDICTIVE MODEL OF HBsAg NEGATIVIZATION IN INACTIVE HBsAg CHRONIC CARRIERS, MAINLY CAUCASIAN

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Background and Aims: HBsAg negativization is the last phase of HBV chronic infection and represents its resolution. The aim of this study was to analyze the frequency of HBsAg negativization in HBV inactive carriers (HBV-IC) and to develop a predictive model of it.
Methods: 297 HBV-IC were studied, 51% males, 86% Caucasians, 42% genotype A and 35% genotype D, 67% with liver elastography <6.2 kPa and with a mean age of 45±13 years. HBV-IC diagnosis was based on HBeAg negativity and HBV-DNA <20000 IU/ml, confirmed in several controls. HCV, HDV and HIV coinfection were excluded

in all. Basal determinations of HBV-DNA (RT-PCR, Cobas TaqMan VHB) and quantitative HBsAg (Architect HBsAg; Abbott), with lower limit of detection of 0.05 IU/ml, were performed. Patients were prospectively followed annually or biannually, with a mean follow-up of 52±28 months.
Results: 57/297 (19%) were initially HBV-DNA negative. Mean HBsAg level was 8.852±17.700 IU/ml. During the follow-up, 49 patients (16.5%) became HBsAg-negative. In univariate analysis (Kaplan–Meier), HBsAg negativization was not associated with sex ($p = 0.18$), ALT normal or high values ($p = 0.8$), liver elastography < or ≥6.2 kPa ($p = 0.45$), or race ($p = 0.96$). The probability of HBsAg negativization was significantly higher in patients >30 years ($p = 0.02$), HBV-DNA-negative ($p < 0.001$) and with initial HBsAg <1000 UI ($p < 0.001$). In multivariate analysis, initial HBV-DNA absence (HR 2.37 [95% CI 1.34–4.19], $p = 0.03$) and basal HBsAg <1000 IU/ml (HR 49.4 [95% CI 6.76–361.02], $p < 0.001$) were independently associated with the probability of HBsAg clearance. The presence or absence of these factors allowed to establish three groups of patients with a significant different probability of HBsAg negativization at 5 years: In those without any favorable factor ($n = 151$) such probability was 0%, in those with one factor ($n = 101$) 27.2%, and in patients with both of them ($n = 45$) 53.5% ($p < 0.001$). Isolated use of HBsAg was also useful to distinguish three groups of patients with different probability of HBsAg negativization at 5 years: 0% in those with HBsAg >1000 UI/ml, 15.2% if HBsAg was between 100 and 1000 IU/ml and 53% when it was <100 IU/ml. However, area under the ROC curve was greater in the model using HBsAg and HBV-DNA (0.88 [0.83–0.92]) than in the one based only in HBsAg (0.84 [0.78–0.89]).
Conclusions: In a series of HBV-IC, mainly Caucasians, the combination of HBsAg cuantification and the presence or absence of HBV-DNA is useful to predict the probability of infection resolution at 5 years.

P0594
CAN SERUM LEVEL OF HEPATITIS B SURFACE ANTIGEN (HBsAg) DIFFERENTIATE HBsAg INACTIVE CARRIER STATE FROM CHRONIC HEPATITIS B?

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Background and Aims: HBeAg-negative hepatitis B virus (HBV) infection exerts both inactive carrier (IC) state and chronic hepatitis B (CHB) which are sometimes difficult to be differentiated. Recently, serum HBsAg level has been introduced to evaluate treatment response to interferon and probably help in diagnosis of hepatitis B clinical stages. We aimed to assess the role of HBsAg level in differentiation of IC and CHB among a group of chronic HBeAg-negative HBV-infected patients.
Methods: A total of 251 HBeAg-negative HBV-infected patients were enrolled. Serum alanine transaminase (ALT), HBV DNA and HBsAg levels were determined for each patient. Liver histology was evaluated by liver biopsy using Knodell scoring system. HBV DNA and HBsAg levels were assessed using COBAS TaqMan HBV test and HBsAg II quant assay, respectively.
Results: A total of 243 HBeAg-negative HBV-infected patients including 139 ICs and 104 CHB patients were evaluated. All HBV isolates were identified as genotype D. HBV DNA and HBsAg levels were significantly higher in CHB patients than in ICs. HBV DNA quantification with cutoff value 2,000 IU/mL for diagnosis of CHB had 99.0% sensitivity and 74.1% specificity. A cutoff value of HBsAg level at 1,000 IU/mL was more reliable for diagnosis of CHB with 82.7% sensitivity and 66.2% specificity than other HBsAg level cutoffs. Combination of HBV DNA and HBsAg levels did not increase diagnostic performance of HBV DNA level for differentiation of IC and CHB stages. There was a positive correlation between HBV DNA and HBsAg levels in both IC ($r = 0.43$, $P < 0.001$) and CHB ($r = 0.42$, $P < 0.001$) groups.
Conclusions: Single point HBsAg quantification did not have enough sensitivity and specificity for diagnosis of HBV clinical stages.

P0595
HIGH FREQUENCY OF ACTIVE HCV INFECTION AMONG SEROPOSITIVES IN WEST AFRICA AND EVIDENCE FOR MULTIPLE TRANSMISSION PATHWAYS

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Background and Aims: Sub-Saharan Africa (SSA) has among the highest global Hepatitis C Virus (HCV) sero-prevalence estimates. However, reports suggesting high rates of serologic false positives and low levels of detectable viremia has led to uncertainty regarding the burden of active HCV infection in this region. Additionally, little is known about the predominant transmission risk factors and mechanisms in this region. The aims of this study were to determine the frequency of active infection among persons who screened positive for HCV infection and identify risk factors for HCV infection.
Methods: Between May 2013 and January 2014 we recalled 363 blood donors [180 rapid screen assay (RSA) (Accu-Tell HCV) positive and 183 RSA negative at time of donation] to identify the level of active infection and risk factors at Komfo Anokye Teaching Hospital in Kumasi, Ghana. Participants had blood drawn for serologic and virologic testing (HBVsAg Abbott Architect CIA, HIV 4th generation Ab/Ag test, the HCV Advia Centaur HCV CIA, and Abbott RealTime PCR assay for HCV RNA quantitative levels). HCV genotypes were determined by the generated NS5b sequences (SuperScript® VILO™ cDNA Synthesis Kit). A questionnaire on demographics and risk factors was administered.
Results: The frequency of active infection varied based on serologic testing results, but was overall high. In subjects with a positive CIA Serologic Antibody Assay [Signal to Cut-off ratio (S/C) >1], the rate of active viremia was 74.4%, and increased to 88% among the individuals with a CIA S/C ≥11. Individuals were predominantly infected with genotype 2, and the median viral load among actively infected individuals was 5.75 log cp/ml. Blood donors from the northern and upper regions of Ghana had substantially higher risks of infection compared to those from the middle belt. Individual level Odds ratio statistical significant risk factors included: traditional circumcision (3.8), home birth (2.0), tribal scarring (2.2) and HBV co-infection (2.7). See Table 1.
Conclusions: Among serologically confirmed cases, active infection rates were high. Appropriate testing algorithms should be widely implemented to define the true HCV burden in SSA. These data also suggest that several transmission modes, particularly

those associated with cultural skin-piercing practices, are likely contributing to the current HCV epidemic in Ghana, and the distribution of these practices may result in regional variation in prevalence.

Table 1. Multivariable logistic regression model for HCV seropositivity

Variable	OR (95% CI)	*p*-value
Region of Origin		<0.001
Northern vs. Ashanti	6.64 (2.44–18.23)	<0.001
Other vs. Ashanti	1.15 (0.33–3.93)	0.83
Upper vs. Ashanti	18.69 (8.27–42.22)	<0.001
Education level		0.01
Nil/Primary vs. Tertiary	0.42 (0.16–1.11)	0.08
JHS/MSLC/SHS/Tech vs. Tertiary	0.29 (0.12–0.66)	0.003
Marital status	2.31 (0.80–6.70)	0.06
Married vs Single	0.93 (0.45–1.92)	0.83
Other vs. Single	8.04 (1.28–50.35)	0.03
HBsAg positive	3.82 (1.08–13.53)	0.04
Circumcision		0.01
None vs. Hospital	3.46 (1.31–9.12)	0.01
Traditional vs. Hospital	3.26 (1.47–7.21)	0.004

OR, covariate-adjusted odds ratio. CI, confidence interval for estimate.

P0596
ASSOCIATION OF HEPATITIS E VIRUS AND CRYOGLOBULINEMIA

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Background and Aims: Several extrahepatic manifestations have been observed in the context of acute or chronic hepatitis E virus infections. Recently a case indicated an association between HEV infection and cryoglobulinemia (Pischke et al. Lancet Inf. Dis. 2014), but this observation still needs to be confirmed and clarified.
Methods: Stored serum samples of 68 German patients with cryogluobulinemia were retrospectively tested for anti HEV IgG (Wantai assay). Seroprevalence rates were compared in patients with essential cryoglobulinemia (n = 33) and in patients with cryoglobulinemia secondary to various underlying conditions (n = 35) using chi square test.
Results: Within the group of patients with essential cryoglobulinemia 46% (n = 15) tested positive for anti HEV IgG, while in the group of patients with different underlying conditions 23% (n = 8) tested positive (p = 0.043).
Conclusions: Patients with essential cryoglobulinemia tested positive more frequently for anti HEV than patients with secondary cryoglobulinemia due to well defined causes. This indicates that development of cryoglobulinemia of unknown origin is a relevant extrahepatic manifestation of hepatitis E.

Table 1. Characteristics of patients

	Essential cryoglobulinemia (n = 33)	Cryoglobulinemia of defined origin (n = 35)	p-value
Anti HEV IgG positive	15 (46%)	8 (23%)	0.043
Male	12 (36%)	14 (40%)	ns
Age in years, range (mean±SD)	37–83 (61.7±13.2)	36–76 (57.4±10.8)	ns
OD value of anti HEV IgG	0.004–3.239 (0.686±1.035)	0.000–3.205 (0.326±0.758)	ns

P0597
THE ROLE OF GENOTYPE, PRE-CORE, BASAL CORE PROMOTER AND PRE-S MUTATIONS OF HBV IN PATIENTS OF HEPATOCELLULAR CARCINOMA WITH HEPATITIS C AND OCCULT HEPATITIS B

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Background and Aims: HBV coinfected with chronic hepatitis C subjects were found to increase the risk to develop advanced liver disease later in their life. In chronic HBV patients, some virological factors such as genotype C, preS deletion and core promoter/precore mutations are found to increase the risk of HCC. However, little was known about the role of the virological risk factors of HBV in chronic HCV patients with occult HBV.
Methods: One hundred and eighty-one HBsAg-negative, HCV-Ab positive HCC patients (group A), 153 HBsAg-negative, HCV-Ab positive chronic hepatitis patients (group B), and 20 HCC subjects with both positive HBsAg and HCV-Ab (group C) were enrolled. The preS, core promoter/precore region and S gene were amplified by nested PCR and direct-sequenced. Viral phylogenetic analysis was also performed.
Results: The occult HBV infection was found for 20.4% (37/181) in the group A, and 34.0% (52/153) in group B. In addition, the genotype C (P = 0.019), BCP mutations (P = 0.004) and 1858 mutation (P < 0.001), 1896 mutation (P = 0.032) were more common in chronic HCV patients with HCC. PreS1 deletion (P = 0.033), BCP (P = 0.014) and precore 1896 mutation (P = 0.008) were more common in chronic HCV patients with overt than occult HBV.
Conclusions: In patients with chronic HCV and occult HBV infection, HBV genotype C, BCP, 1858 and 1896 mutations seemed to be associated with the development of HCC.

P0598
NATURAL HISTORY OF CHRONIC HEPATITIS B INFECTION IN THE GAMBIA, WEST AFRICA: A LONGITUDINAL POPULATION-BASED STUDY

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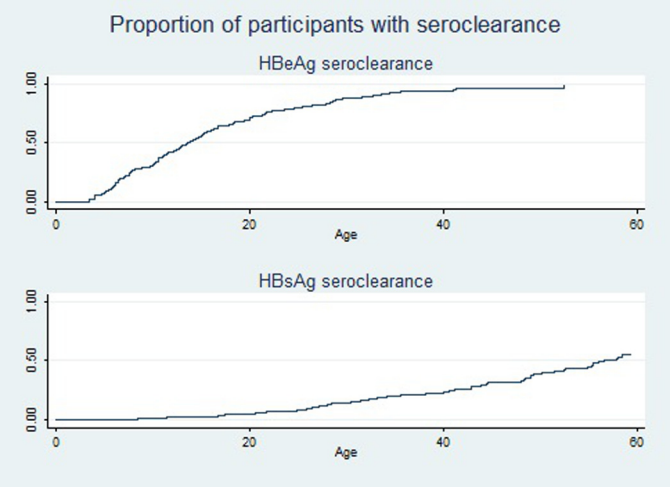
Background and Aims: The natural history of chronic hepatitis B (CHB) infection in sub-Saharan Africa is poorly documented. This study describes the natural history of CHB in The Gambia, West Africa.
Methods: An open community cohort of treatment-naïve CHB carriers was recruited from rural villages in the West Kiang district, The Gambia between 1974 and 2008. The cohort was used to estimate the rates of hepatitis B e (HBeAg) and surface antigen (HBsAg) clearance and incidence of hepatocellular carcinoma (HCC). In 2012–2013 we invited members of the cohort for a comprehensive liver assessment to estimate the prevalence of chronic liver disease as part of the PROLIFICA study.
Results: 405 chronic carriers were identified in 10 sero-surveys, and the median length of follow up was 28.4 years. Annually, 7.4% (95% CI: 6.3–8.8) and 1.0% (95% CI: 0.8–1.2) of the carriers cleared HBeAg and HBsAg, respectively (Figure). The incidence

of HCC was 55.5/100,000 carrier-years (95% CI: 24.9–123.5). The median age of the CHB carriers assessed in the 2012–13 survey (n = 301) was 38 years, 43.2% were male, 5.6% (95% CI: 3.4–9.1%) had significant fibrosis and 3.7% (95% CI: 2.0–6.5%) required antiviral therapy according to the EASL guidelines. Having an HBsAg-positive mother was associated with higher viral load and ALT levels over time, liver fibrosis, requiring antiviral treatment, and HCC. Among chronic carriers, 63.0% (95% CI: 47.0–74.1%) of the cases requiring antiviral therapy were attributable to perinatal transmission.

Conclusions: The incidence rate of HCC in CHB carriers in The Gambia was higher than Europe but lower than East Asia. Positive maternal HBsAg, a proxy for perinatal mother-to-infant transmission, was associated with an increased risk of liver fibrosis and HCC. Prevention of perinatal HBV transmission through the timely administration of hepatitis B vaccine to infants within 24 hours after birth may reduce the disease burden in sub-Saharan Africa.



P0599
ROLE OF NON-HEPATOTROPIC VIRUSES IN ACUTE SPORADIC VIRAL HEPATITIS IN ADULTS: SHOULD THE SCREENING STRATEGIES BE CHANGED?

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Background and Aims: Acute viral hepatitis (AVH) is mostly caused by Hepatitis E (HEV) and hepatitis A (HAV) viruses in India. The etiology of AVH has been dynamically changing during the recent years and the viral etiology of acute event in Acute on chronic liver failure (ACLF) patients has rarely being explored.

Methods: We hypothesized that the viral causes of acute hepatitis in AVH and ACLF group would differ hence a retrospective analysis of 295 adult patients of acute sporadic viral hepatitis with the criteria: <3 weeks onset of fever or jaundice, serum alanine transaminase (ALT) 10 times the upper limit of normal and with a definite established viral etiology was done. Patients were further divided into two groups, only AVH and ACLF as per clinical criteria. Serum samples were tested for hepatitis A through E, cytomegalovirus (CMV), and Epstein-Barr virus (EBV).

Results: Viral etiology profile was, HEV in 155 (52.5%), HAV in 43 (14.5%), HBV in 35 (11.8%), HCV in 1 (0.3%), mixed viral etiology in 30 (10.1%) and non-hepatotropic viruses (CMV and EBV) in 31 (10.5%). 206 patients (69.8%) were only AVH and 89 (30.1%) were ACLF. HEV was the commonest cause of infection in both the groups: AVH (n = 95, 46.1%) and ACLF (n = 60, 67.4%) as compared to HAV: AVH (n = 36, 17.4%), ACLF (n = 7, 7.8%), [p < 0.001]. 23 (7.7%) patients died on follow up, mortality was higher in ACLF group (12/89, 13.5%) than in AVH group (4/206, 1.94%) [p < 0.05]. The mortality was higher when HEV was the cause of acute event in ACLF group (20/23, 86.9%). In AVH mixed infection were more common as compared to ACLF (27 vs. 3, p < 0.001). In mixed viral etiology, HBV + HEV and HAV +HEV dual infection was seen in 11 (0.03%), whereas HBV +HAV was seen in 8 (0.02%) cases. Non-hepatotropic viruses like CMV and EBV contributed significantly higher infections in AVH as compared to ACLF (14% vs 2.2%, p = 0.002) patients. Most cases of EBV and CMV infections presented with only abnormal ALT levels and were not associated with any lymphadenopathy (87%).

Conclusions: Non-hepatotropic viruses like CMV and EBV contribute significantly in the etiology of sporadic AVH in adults. Apart from screening for hepatotropic viruses (A-E), all suspected acute hepatitis patients must be screened for non-hepatotropic viruses as well.

P0600
PREDICTIVE FACTORS OF CIRRHOSIS, HEPATIC DECOMPENSATION AND HEPATOCELLULAR CARCINOMA IN PATIENTS WITH CHRONIC HEPATITIS DELTA

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Background and Aims: Hepatitis Delta Virus (HDV) infection causes more severe chronic liver disease than isolated Hepatitis B virus (HBV) infection, although it remains largely under-diagnosed and under-evaluated in most countries. Factors associated with worse prognosis have not been fully evaluated in France.

Methods: HDV-infected patients were identified from the French National Reference Centre database. Epidemiological clinical and virological data, including HDV viral load and HDV genotyping, were collected on a standardized form, from initial diagnosis to the last recorded follow-up.

Results: 1112 HDV/HBV co-infected patients were included in the study. The mean age was 37±10 years. Patients were predominantly male (69%) and migrants (86%). 46% had a history of past or persistent excessive alcohol consumption, and 37% were overweight. 88% showed detectable HDV viral load (median value: 165,000 copies/mL). At first referral 28% of the patients showed histological cirrhosis, 15% one episode (or more) of hepatic decompensation and 2.7% hepatocellular carcinoma (HCC). During the median follow-up of 4.4 years [1.4–8.9], 20%, 10% and 6.5% of the patients developed cirrhosis, liver decompensation and HCC, respectively. Overall, the 10-year risk of cirrhosis, decompensation, HCC was 40%, 31%, and 15%, respectively.

In multivariate analysis, older age and elevated GGT were independent factors associated with cirrhosis (HR = 1.03 and 2.1 respectively), hepatic decompensation (HR = 1.04 and 2.6 respectively) and HCC (HR = 1.10 and 3.7 respectively). Cirrhosis was also associated with elevated AST (HR = 2.2) and less frequent in sub-Saharan African patients (HR = 0.76) than in European and Asian patients. Decompensation was associated with positive HDV RNA at last evaluation (HR = 2.2), low platelet count <100,000/mm^3

(HR = 3.8), and prothombin time <60% (HR = 6.4), and HCC with BMI >30 kg/m^2 (HR = 2.5) and prothombin time <80% (HR = 6.2).
Conclusions: In chronic HDV-infection, older age and severity of the disease are associated with cirrhosis and complications. Persistent replicative HDV infection is associated with decompensation. Unexpectedly, cirrhosis is less frequent in sub-Saharan African patients than in non African patients. Obesity is an independent factor associated with HCC related to HDV infection.

P0601
ACOUSTIC RADIATION FORCE IMPULSE IN HEPATITIS B VIRUS INACTIVE CARRIERS – A COMPARISON WITH OTHER CONDITIONS
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Background and Aims: Inactive hepatitis B virus (HBV) carriers are defined as HBs antigen positive, HBe negative patients that have persistently normal transaminase levels and low or undetectable HBV DNA levels. Usually they are not subjected to liver fibrosis evaluation (either invasive or noninvasive) especially if their viral load is below 2000 IU/ml. In this study, we tried to compare the differences between the results of Acoustic Radiation Force Impulse (ARFI) measurements in inactive HBV carriers and in patients with other liver diseases or with no known liver afection.
Methods: 159 patients [43 inactive HBV carriers, 52 randomly selected patients with chronic hepatitis C virus (HCV) infection, 25 patients with ultrasound aspect suggesting fatty liver (FL) but normal transaminase and 39 patients with no evidence of any liver disease] were included in the analysis. We performed 10 consecutive ARFI measurements in the right liver lobe of each patient. In all included patients, interquartile range (IQR) was less than 1/3 of the median of the 10 measurements.
Results: In HBV inactive carriers the mean ARFI result was 1.34±0.34 m/s, in HCV patients 1.84±0.77 m/s, in FL patients 1.02±0.24 m/s, and in healthy patients 1.08±0.21 m/s. ARFI results in HBV carriers are significantly higher than in healthy or FL patients (mean differences: 0.262 m/s, 95% CI 0.118–0.407, p = 0.001 respectively 0.321 m/s, 95% CI 0.141–0.502, p = 0.001). The stiffness in HCV patients was significantly higher than in HBV carriers, as denoted by a speed of propagation of shear waves of 0.502 m/s, 95% CI 0.348–1.037, p < 0.001. Transaminase levels were not significantly different between HBV carriers and healthy patients, but they were significantly higher in FL patients (although normal) than in HBV carriers – mean difference 24.84 IU/l for ALT and 14.77 IU/l for AST, with p = 0.001 and 0.013 respectively.
Conclusions: Liver stiffness measured by ARFI is significantly increased in HBV inactive carriers compared to patients with no liver disease or with fatty liver disease and normal transaminase. This fact is probably related to a mild necroinflammatory process that may be sometimes present in "inactive HBV carriers" and is not reflected by ALT elevation.

P0602
SEROLOGIC PROFILE AND REACTIVATION OF HEPATITIS B IN RHEUMATIC AND INFLAMMATORY BOWEL DISEASE PATIENTS TREATED WITH TNF INHIBITORS BOWEL DISEASE
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Background and Aims: TNF inhibitors (TNFi) are widely used in rheumatologic diseases (RD) and inflammatory bowel disease (IBD). However, these drugs have been associated with an increased risk of reactivation of latent infections. The specific risk of hepatitis B (HB) reactivation remains unclear since the low incidence of that infection in the majority of European countries. Our study aims to evaluate the serologic HB profile of patients with RD and IBD who had undergone TNFi therapy in a single centre; and to assess the incidence of HB reactivation in patients with a serological pattern of past HB infection.
Methods: Retrospective study of HB baseline serologic profile in patients with IBD and RD who have started TNFi therapy at our institution. Clinical signs of liver injury and liver injury blood tests were analysed during the treatment in order to identify cases with reactivation. Reactivation was defined as the detection of AgHBs or DNA-VHB.
Results: We analyzed 484 patients with available data on HB serologic profile, 210 men (43.4%) with a mean age of onset of biological therapy of 38±15 years. 258 patients were diagnosed with RD (100 rheumatoid arthritis, 76 ankylosing spondylitis and 82 other RD); 226 patients suffered from IBD (180 Crohn's disease, 41 ulcerative colitis and 5 indeterminate colitis); 273 patients were simultaneously submitted to immunosuppressive treatment with azathioprine, 6-mercaptopurine, corticosteroids or methotrexate, for at least 1 month. At the beginning of the treatment, the HB serologic profile variations were: negative in 63.4%, 31.0% AbHBs+/AbHBc- and 5.8% AbHBc+ (with or without AbHBs+). The 28 AbHBc+ patients did not differ significantly from the overall population in terms of demographic characteristics, diagnosis, follow-up interval and biological therapy. No patient received prophylactic antiviral therapy during the follow-up period (median 8 years and 80 days) and there was only one case of HB reactivation, a Crohn's disease patient treated with infliximab and azathioprine.
Conclusions: In our cohort, there was one case of HB reactivation, corresponding to 3.5% of AbHBc+ patients treated with TNFi. This study highlights the importance of assessing HB serologic profile at the beginning of immunosuppressive therapy. This strategy allows not only the vaccination of serologic negative patients but also the identification of patients at risk of HB reactivation.

P0603
ASSOCIATION OF PRE-S MUTATION WITH OCCULT HBV INFECTION AND RITUXIMAB-RELATED HBsAg REVERSE SEROCONVERSION IN PATIENTS WITH LYMPHOMA
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Background and Aims: Occult HBV infection (OBI) was observed in over one third of patients with lymphoma and resolved hepatitis B in our previous randomized study. Whether the pre-S mutation/deletion and surface region mutation associated with the negativity of HBsAg was unclear.
Methods: Thirty anti-CD20-positive lymphoma patients negative for HBsAg with HBV viremia (occult HBV infection) and 30 age- and sex-matched HBeAg-negative chronic hepatitis B (CHB) patients were enrolled. HBV pre-S and surface regions were sequenced. Site-directed mutagenesis for the hot spot mutations was performed to generate replication-competent mutant HBV plasmids. The expressions of HBsAg by wild and mutant HBV were validated by western blotting. Mutations within the pre-S/S region from serial samples of the resolved hepatitis B patients with rituximab-related HBV reactivation were also compared.
Results: As compared with CHB patients, multiple mutations within the preS1 I84L/Q, preS2 G30E, and surface regions S114T, T131I/N, A184G/V, F220C/L, C221A/G/Y, I226M/S/V were highly associated with OBI. Pre-S deletion was identified only in one patient with OBI. Interestingly, preS2 G30E mutation decreased the amount of HBsAg

secretion but had no impact on the expression of intracellular HBsAg in vitro. Of the patients with HBV reactivation, all cases had pre-S2 G30E mutation before the reactivation and converted to wild type during the event of HBsAg reverse seroconversion.
Conclusions: Multiple mutations in preS/S regions were observed in lymphoma patients with OBI. Pre-S2 mutation is associated with the secretion of HBsAg. Distinct HBV evolution accounts for the rituximab-related HBsAg reverse seroconversion in lymphoma patients with resolved hepatitis B.

P0604

EVOLUTIONARY CHANGES OF HEPATITIS B VIRUS PRE-S MUTATIONS PRIOR TO DEVELOPMENT OF HEPATOCELLULAR CARCINOMA

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Background and Aims: Hepatitis B virus (HBV) pre-S deletions/mutations have been associated with hepatocellular carcinoma (HCC). However, the evolutionary changes of pre-S mutations prior to HCC diagnosis remain unexplored.
Methods: HBV pre-S sequences were determined in 74 chronic hepatitis B (CHB) patients with HCC and 146 CHB patients who have been followed up for >3 years with no HCC (HCC-free). Samples collected at the following time points were studied: 1–3 years, 4–6 years, 7–9 years and ≥10 years prior to HCC development and at the same time points for the HCC-free group from last follow-up. HBV pre-S mutations were assessed by comparison with 54 wild-type reference sequences retrieved from the NCBI Genbank.
Results: Pre-S deletions were detected in 31.1%, 25%, 48.5%, and 42.8% of HCC patients at 1–3 years, 4–6 years, 7–9 years, and ≥10 years prior to HCC development, respectively. In the HCC-free group, the frequencies of pre-S deletions at the 4 corresponding time points were lower (9.6%, 7.9%, 8.8%, and 17.6%, respectively; with p values of <0.001, 0.036, <0.001, and 0.096, respectively). At 1–3 years prior to HCC development, higher frequencies of pre-S point mutations at 11 codons (codons 4, 27, 51, 54, 60, 62, 100, 125, 137, 166 and 167) were observed in the HCC patients (range: 14.9–75.7%) than in the HCC-free patients (range: 5.5–26%; all $p < 0.05$). Ten out of these 11 pre-S mutations (except codon 4, which emerged at 4–6 years before HCC) existed ≥10 years before HCC. Among these 11 codons, the number of mutated codons with differential distribution between HCC and HCC-free patients increased with time prior to HCC: a significantly higher frequency of mutations was detected in the HCC patients in 2/11 codons at ≥10 years, in 9/11 codons at 7–9 years, and in 10/11 codons at 4–6 years prior to HCC. The total number of HCC-associated pre-S point mutations increased with time prior to HCC and correlated with the time to HCC development ($r = 0.151$, $p = 0.042$), while no correlation was observed in the HCC-free group ($r = 0.073$, $p = 0.398$). Multivariable logistic regression analysis showed that pre-S deletions and point mutations at codons 27, 51 and 167 were independent factors associated with increased HCC risk (all $p < 0.05$).
Conclusions: High prevalence and cumulative evolution of pre-S mutations preceding HCC development confirmed the carcinogenic role of pre-S mutations. The effect of individual pre-S mutations on HCC development warrants further studies.

P0605

GENOTYPE SPECIFIC VARIATION IN THE BASAL CORE PROMOTER AND PRE CORE REGIONS IMPACTS HBeAg LEVELS DURING IMMUNE CLEARANCE DISEASE

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Background and Aims: Basal core promoter (BCP) and precore (PC) mutations first emerge prior to HBeAg seroconversion and have been associated with lower HBeAg expression, liver fibrosis and HCC. GS-US-174-0103 is a phase 3 study evaluating tenofovir in HBeAg-positive chronic hepatitis B (CHB). The aim of the current study was to use next generation sequencing (NGS) to evaluate the prevalence and quantitative frequency of key mutations within the BCP and PC regions and evaluate their impact on baseline virological profiles within the 103 cohort.
Methods: Illumina MiSeq full genome NGS was performed on a subset of 94 baseline samples from patients enrolled in study 103, using a threshold for detection of 1%. The results of NGS were analysed against baseline demographic, clinical, and serological data including HBV DNA, HBsAg, and HBeAg levels. In the first instance only mutations in the BCP and PC regions were considered.
Results: Data were available for 94 patients (median: age 32 yrs, 69% male, HBV DNA 8.43 log10 IU/mL, HBeAg 3.30 log10PEIU/mL, HBsAg 4.71 log10 IU/mL, ALT 137.5 IU/mL, METAVIR fibrosis stage 3). BCP and PC variants were detectable in 34% and 15% of patients using population sequencing respectively vs. 43% and 41% using NGS ($p < 0.001$). Differences were seen in the genotype (Gt) distribution of mutations within the BCP and PC regions (Table 1).
The presence of mutation at A1762 and G1764 was associated with significantly lower HBeAg expression at baseline ($p < 0.0001$ for both). Presence of the G1896A mutant alone was not associated with HBeAg expression. HBsAg levels ($p = 0.002$), A1762T ($p = 0.002$) and T/C1858G ($p = 0.02$) were independently associated with HBeAg levels at baseline (model $p < 0.0001$; R2= 0.31). Baseline HBsAg levels were independently associated with HBV DNA ($p < 0.0001$), genotype ($p = 0.005$) and A1762T and G1764SA ($p < 0.0001$ for both) (model $p < 0.0001$; R2= 0.48).

Table 1. Selected BCP/PC mutations detected by NGS

	n	BCP			PC
		A1762T	G1764A	T/C1858G	G1896A
All	94	34 (36%)	34 (36%)	13 (11%)	39 (41%)
Gt A	26	5 (19%)	6 (23%)	2 (8%)	0
Gt B	19	3 (16%)	3 (16%)	0	15 (79%)
Gt C	21	18 (86%)	19 (90%)	8 (38%)	9 (43%)
Gt D	28	8 (29%)	6 (21%)	1 (4%)	15 (54%)

Conclusions: Clinically important HBV variants are frequently present in treatment naïve patients during the immune clearance phase of CHB prior to the initiation of therapy, often below the threshold of detection by population based Sanger sequencing. These include mutations associated with increased risk of disease progression. Our data suggest that molecular differences in the HBV genome, both within and between genotypes may contribute

to variations in disease status among patients and illustrates the power of NGS for detecting clinically important variants.

P0606

TNF-ALPHA SIGNIFICANTLY INHIBITS HEPATITIS E VIRUS REPLICATION

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Background and Aims: Several previous reports suggest an involvement of TNF-alpha in hepatitis E virus (HEV) infection. Polymorphisms in the promotor region as well as serum level of TNF-alpha have been identified to influence clinical outcome of HEV. We aim to characterize the influence of TNF-alpha on viral replication in tissue culture.
Methods: We assessed viral replication under treatment with TNF-alpha by transfection of the hepatoma cell-line HepG2 with a previously described genotype 3 clone of HEV (Kernow p6), which harbours a gaussia-luciferase gene in the ORF2 coding region. Additionally, we performed co-treatment experiments with already approved anti-TNF-alpha drugs as well as ribavirin, a potent inhibitor or HEV-replication. We are currently confirming our results by using the full-length clone of Kernow by addressing viral production with detection of HEV-RNA as well as capsid antigen.
Results: TNF-alpha inhibited HEV-replication in a dose-dependent manner with an IC50 of 50 μg/ml. Addition of an TNF-alpha receptor inhibitor (Etanercept) as well as an TNF-alpha blocking antibody (Certulilumab) completely abolished the antiviral activity of TNF-alpha. Co-treatment with TNF-alpha as well as ribavirin revealed an additive antiviral effect.
Conclusions: As suggested by previous clinical observations TNF-alpha directly influences viral replication in vitro. Patients which receive anti-TNF-alpha drugs should be carefully evaluated for liver enzymes and an elevation should prompt for testing of HEV-RNA, in particular.

P0607

PREVENTION OF VERTICAL TRANSMISSION OF HEPATITIS B: AN EVALUATION OF ADHERENCE RATES AND RISK FACTORS FOR FAILURE TO PROVIDE IMMUNOPROPHYLAXIS THERAPY IN A SINGLE AUSTRALIAN CENTRE

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Background and Aims: Immune prophylaxis therapy (IPT) is well established in decreasing perinatal transmission of hepatitis B virus (HBV) and is a key strategy to reduce the prevalence of chronic hepatitis B (CHB) worldwide. Universal guidelines recommend administration of HBV immunoglobulin (HBIg) and HBV vaccine within 12 hours of birth. Immunisation adherence rates and risk factors for failure to provide timely IPT to babies born to CHB mothers remain poorly defined. We conducted a retrospective cohort study to determine compliance with IPT guidelines in routine obstetric practice at Monash Health, Australia.
Methods: 451 mothers with CHB delivered 451 live born infants at Monash Health between 2008 and 2013. There were 3 stillbirths and 3 sets of twin livebirths. Demographic and disease data, referral rates for specialist HBV care, neonatal and maternal morbidity outcomes and timing of HBIg and HBV vaccine administration were extracted from hospital records. Multivariate logistic regression analysis was utilized to identify the risk factors associated with failure to provide timely administration of IPT.
Results: 47.23% of CHB mothers were diagnosed on routine antenatal screening. Mean maternal age was 30.37±5.25 years and mean gestational age was 38.99±2.50 weeks. 55.67% of women received antenatal specialist care for CHB and 4.29% commenced prophylactic antiviral therapy in the third trimester. 79.82% of newborns received HBIg within 12 hours and 8 babies received no HBIg prior to discharge (1 due to withdrawal of parental consent). 97.12% of newborns received HBV vaccine within 12 hours and 1 baby received no HBV vaccination prior to discharge. On multivariate analysis, antenatal care provided by an Obstetrician and or a Specialist for CHB was associated with timely administration of HBIg (OR 1.64, $p = 0.038$, CI:1.03–2.61). The requirement for an interpreter at birth, maternal age and parity, post-partum complications, gestational age and neonatal morbidity were not significantly related to delayed administration of HBIg. There were no predictors for timely HBV vaccine.
Conclusions: The efficacy of IPT in the prevention of HBV vertical transmission has been well documented. However, the adherence rate to these recommendations remains sub-optimal especially for timely HBIg administration in routine obstetric practice. Targeted clinical interventions to improve HBIg adherence rates and serologic follow-up of infants born to CHB mothers is recommended.

P0608

CAN MBL2 GENOTYPES AND ITS POLYMORPHISM PREDICT THE OCCURRENCE OF CIRRHOSIS AND HEPATOCELLULAR CARCINOMA?

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Background and Aims: Mannose binding lectin (MBL2) is a central component of the innate immune response and thus polymorphisms are reported to play a crucial role in outcome of hepatitis B virus (HBV) infection. The study was designed to ascertain the association of MBL2 genotypes (codon 52, 54, 57 and promoter −550, −221) and its serum expression levels with reference to different stages of HBV infection in patients of Chronic hepatitis B, cirrhosis of liver and hepatocellular carcinoma.
Methods: The study included 150 patients of liver diseases in various stages of HBV infection and 100 healthy age and sex matched individuals who were negative for hepatitis B infection served as controls. All the samples were processed for quantification of MBL levels using commercially available ELISA kits as per the manufacturer instruction [BIOPORTO Diagnostics, Denmark]. The MBL2 gene polymorphisms (codon 52, 54, 57 and promoter −550, −221) were identified by polymerase chain reaction sequence specific primer method.
Results: Individuals without infection had highest levels of serum MBL2 (1388.74±2697.57 ng/ml) when compared with the individuals with infection especially patients with advanced disease possessing lowest levels (380.35±175.10 ng/ml; p value 0.001) (Figure 1). A clear association between *mbl2* genotype and HBV infection outcome was noted in these groups.
Codon 54 mutation was significantly associated with hepatitis B related decompensated cirrhotic patients [p = 0.001; OR = 3.31 (1.53–7.14)] and hepatocellular carcinoma (HCC) [p = 0.009; OR = 3.00 (1.28–6.99)] compared to controls. Promoter −221, low expression genotype CC (X) rate was high in chronic hepatitis B, decompensated cirrhosis, and HCC patients [p = 0.01, OR = 2.66 (1.190–5.935); p = 0.001, OR = 3.61 (1.641–7.979) and p = 0.01, OR = 3.05 (1.278–7.320)] compared to controls.
Conclusions: The results provide preliminary evidence that inheritance of codon 54 genotypes and promoter −221 genotype X associated with low MBL level substantially determine the outcome

of HBV infection and predicts the likelihood of cirrhosis and hepatocellular carcinoma.

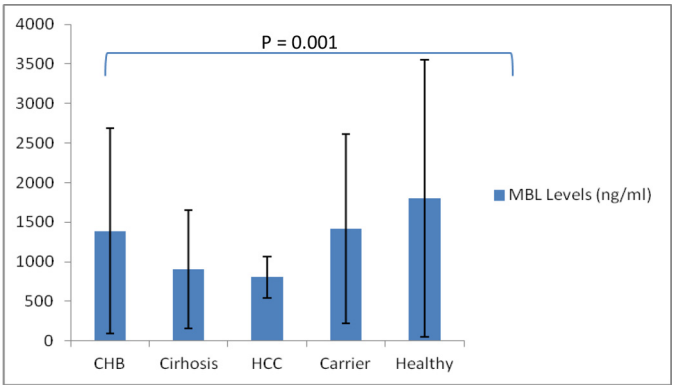

Figure 1. Distribution of serum MBL2 levels.

P0609
RISK PREDICTION OF NON-CIRRHOTIC HEPATOCELLULAR CARCINOMA IN PATIENTS WITH CHRONIC HEPATITIS B VIRUS INFECTION

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Background and Aims: One-third of chronic hepatitis B (CHB) related hepatocellular carcinoma (HCC) patients are non-cirrhotic. This study aimed to investigate the risk factors of HCC in those non-cirrhotic CHB patients
Methods: This analysis included hepatitis B surface antigen (HBsAg)-seropositive participants from the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in HBV (R.E.V.E.A.L.-HBV) cohort. Patients with evidence of cirrhosis at entry were excluded. Cirrhosis and HCC were ascertained through regular follow-up ultrasonography, linkage with national health database, and medical chart reviews. Factors included age, gender, HBV e antigen (HBeAg) status, serum levels of HBV DNA, quantitative serum HBsAg levels, Co-infection with HCV, alanine aminotransferase (ALT), habits of smoking, alcohol consumption, family history of HCC, body-mass index, and diabetes mellitus history were analyzed between LC related HCC and non-LC related HCC patients. Multinomial logistic regression was applied for risk factors investigation.
Results: There were 187 patients developed HCC in 3776 CHB patients, 66 of them were non-cirrhotic HCC patients. Older age (>50 vs. <50 year-old, OR: 4.42 [2.15–9.06], P = 0.000), family history of HCC (OR: 3.03 [1.38–6.68], P = 0.006), cigarette smoking habit (OR: 2.02 [1.05–3.86], P = 0.035), alcohol consumption habit (OR: 2.17 [1.16–4.05], P = 0.015), elevated ALT (15–45 vs. <15 U/L, OR: 2.263 [1.279–4.003], P = 0.005; >45 vs. <15 U/L, OR: 3.431 [1.520–7.744], P = 0.003), HBV DNA $>10^6$ copies/mL in HBeAg-seronegative patients (OR: 2.83 [1.081–7.406], P = 0.034 vs. HBeAg-seronegatives with HBV DNA $<10^6$ copies/mL), or HBeAg positivity (OR: 3.679 [1.791–7.558], P = 0.000) are independent risk factors for non-cirrhotic HCC in CHB patients.
Conclusions: Smoking, alcohol drinking, family history of HCC, abnormal LFT, HBV DNA $>10^6$ copies/mL and HBeAg seropositive status are predictors of non-cirrhotic HCC in CHB patients

P0610
DYNAMIC PREDICTION OF INACTIVE CHRONIC HEPATITIS B USING REPEATED HBsAg AND HBV DNA LEVEL MEASUREMENTS THROUGH LONG-TERM FOLLOW-UP

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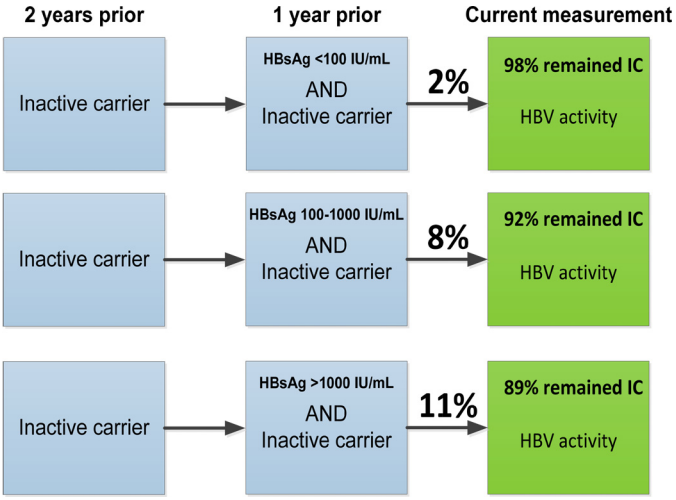
Background and Aims: A single-point hepatitis B surface antigen (HBsAg) level below 1,000 IU/mL combined with low HBV DNA has been shown to identify inactive carriers (IC) after 1 year of follow-up. Our aim was to evaluate the performance of repeated HBsAg measurements through long-term follow-up.
Methods: In this retrospective cohort study conducted at 8 tertiary care centres 293 treatment-naïve non-cirrhotic HBeAg-negative patients with a normal ALT and HBV DNA <20,000 IU/mL were included. HBsAg, HBV DNA and ALT levels were measured at each visit during a median follow-up of 8 years (range 4–9). Patients were defined IC in case of HBVDNA <2,000 IU/mL and persistent normal ALT during a complete follow-up year. A fluctuation >2,000 IU/ml and/or abnormal ALT was defined as HBV activity. Dynamic regression analysis was used to study changes in HBsAg levels and HBV phase.
Results: Of 293 patients, 224 (76%) were IC at inclusion. Mean age was 43±13 years and HBV genotype A/B/C/D was present in 39/42/33/104 patients. Patients with activity in one year and a persistently normal ALT and HBV DNA <2,000 IU/mL during the subsequent year had a 40% chance to have activity again in the year thereafter. By dynamic analysis, the probability to remain IC in the next year given HBsAg levels of <100, 100–1,000 or >1,000 IU/mL was 96%, 86% and 81% (p < 0.001). IC during 2 consecutive years was predictive of IC in the next year, still 10% of patients with HBsAg >100 IU/mL showed disease progression (figure). A single-point HBsAg <100 IU/mL with HBV DNA <2,000 IU/mL could predict IC throughout long-term follow-up with a specificity of 97% and a positive predictive value (PPV) of 95%, while a HBsAg <1,000 IU/mL combined with HBV DNA <2,000 IU/mL had a specificity of 88% and a PPV of 90% for the total cohort. The combined rule of HBV DNA with HBsAg <1,000 IU/mL performed well in HBV genotype D patients (PPV 95%, specificity 92%), still HBsAg levels <100 IU/mL were superior (PPV 99%, specificity 99%). Patients with HBsAg <100 IU/mL also had a high chance of HBsAg loss (HR = 5.1 versus 100–1000 IU/mL, 95% CI: 1.9–13.5, p < 0.001). In those patients with a HBV DNA >2,000–<5,000 IU/mL and a HBsAg decline of ≥0.5 log IU/mL one year prior, 62% became and remained IC.
Conclusions: Both HBsAg levels and declines can be used to identify IC patients. HBsAg levels are predictive of remaining in the IC phase and should be used to define HBV phases.



P0611

TRAJECTORIES OF HEPATITIS B SURFACE ANTIGEN AND ASSOCIATIONS WITH HEPATOCELLULAR CARCINOMA, LIVER CIRRHOSIS, AND HBsAg SEROCLEARANCE AMONG CHRONIC HEPATITIS B CARRIERS WITH LOW VIRAL LOADS

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Background and Aims: Chronic hepatitis B virus (HBV) carriers with low viral loads have significantly decreased risk for hepatocellular carcinoma (HCC) and liver cirrhosis, and are more likely to reach HBsAg seroclearance. Previous studies have shown that baseline serum hepatitis B surface antigen (HBsAg) levels can help to predict HBV-related outcomes, but the role of long-term trajectories of quantitative HBsAg is still unclear. This study aims to examine the role of HBsAg trajectories in predicting HCC, cirrhosis, and HBsAg seroclearance among chronic HBV carriers with low viral loads.
Methods: A total of 1204 individuals from the community-based REVEAL-HBV cohort were included in this study. Participants had viral loads <10,000 copies/mL with normal ALT, and were HBeAg-seronegative, anti-HCV seronegative, and free of cirrhosis at study entry. All participants had >2 measurements of quantitative HBsAg, and trajectories were measured by calculating the decline in HBsAg levels per year of follow-up. The median decrease, 0.059 log iu/mL per year, was used as a cut-off.
Results: Among 1204 individuals with HBV DNA <10,000 copies/mL, the mean (range) baseline HBsAg level was 2.06 (−2.0–4.8) log iu/mL. Compared to those with baseline HBsAg levels <2 log iu/mL, those with HBsAg levels ≥2 log iu/mL had similar rates of HBsAg decline during follow up (P = 0.33). Compared to those without significant declines in HBsAg levels (<0.059 log iu/mL per year) during follow-up, individuals with declines in HBsAg levels ≥0.059 log iu/mL per year had adjusted rate ratios (95% CI, p-value) for HCC, liver cirrhosis, and HBsAg seroclearance of 1.68 (0.66–4.26, p = 0.28), 0.78 (0.48–1.29, p = 0.33), and 1.86 (1.49–2.32, p < 0.001), respectively. Furthermore, compared to those with baseline HBsAg levels ≥100 iu/mL, those with baseline HBsAg levels (iu/mL) and follow-up declines (log iu/year) of <100/<0.059, and <100/≥0.059 had adjusted HBsAg seroclearance rate ratios (95% CI, p-value) of 4.44 (3.43–5.75, p < 0.001), and 7.85 (5.85–10.55, p < 0.001), respectively.
Conclusions: Among chronic hepatitis B patients with low viral loads, decreases in HBsAg levels did not further significantly reduce rates of HCC or cirrhosis, but did significantly predict HBsAg seroclearance. Further clarification of the role of long-term quantitative HBsAg trajectories in the natural history of chronic hepatitis B infection is needed.

P0612

INTERFERON-GAMMA-INDUCIBLE PROTEIN 10 PROMOTER POLYMORPHISM RELATED WITH END-OF-TREATMENT HBsAg LEVELS AND HAD COMPLEMENTARY ROLE IN PREDICTION OF SUSTAINED VIROLOGIC RESPONSE TO PEGINTERFERON IN HBeAg-NEGATIVE CHRONIC HEPATITIS B: MULTICENTER STUDY

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Background and Aims: Interferon-γ-inducible protein 10 kDa (IP-10) has an important role for immune regulation. In chronic hepatitis B (CHB) infection, pre-treatment IP-10 levels are associated with HBeAg loss after Pegylated interferon alfa (PegIFN) therapy. The single nucleotide polymorphism (SNP) G-201A in the promoter of IP-10 gene up-regulates IP-10 expression and is associated with disease progression in CHB infection. This study aimed to determine the impact of the SNP G-201A in IP-10 gene on treatment in HBsAg-negative patients treated with PegIFN.
Methods: 115 patients with HBeAg-negative CHB infection treated for 48 weeks with PegIFN from 13 hospitals were prospectively enrolled. The G-201A SNP in promoter of IP-10 gene was genotyped. Response to treatment was assessed at 24 weeks post-treatment and defined as HBV-DNA <2,000 IU/ml.
Results: Eighty-five patients (81.7%) had HBV genotype C infection and five patients (4.6%) had liver cirrhosis. Fifty-four (55.7%) and six (6.3%) patients achieved virologic remission and HBsAg loss at 24 weeks after therapy, respectively. At week 48, HBV-DNA <1 log IU/ml predicted 80% of sustained virologic remission. Age, sex, genotype, HBsAg level and ALT at week 48 were not predictor for virologic response. However, this study found G-201A SNP of IP-10 gene was related to HBsAg levels during treatment period. Patients with non-GG genotype had significant lower HBsAg level at end-of-treatment than GG genotype (p = 0.002) and trended to sustain suppression of HBsAg level after treatment (Figure 1). Eighty-seven patients (93.5%) had HBsAg level <5,000 IU/ml at week 48 and in this group, patients who had non-GG significant achieved more virologic remission than GG genotype (78.9% vs 50.9%, p = 0.033). By multivariate analysis, only −201 IP-10 non-GG genotype was associated with sustained virologic response (adjusted OR: 3.62, 95% CI: 1.06–12.28, p = 0.039). Median rate of HBsAg reduction from pretreatment level to week 48 was not different between responders (−2.87 log IU/ml) and non-responders (−3.19 log IU/ml) (p = 0.08). The SNP −201 was not associated with HBsAg loss and ALT normalization after therapy.
Conclusions: In HBeAg-negative CHB patients treated with PegIFN, combination IP-10 promoter polymorphism with end-of-treatment

HBsAg level strongly predict sustained virologic response. Non-GG genotype of IP-10 gene promoter polymorphism was linked to low HBsAg level at end-of-therapy. Further study on immunopathophysiology is needed to elucidate this association.

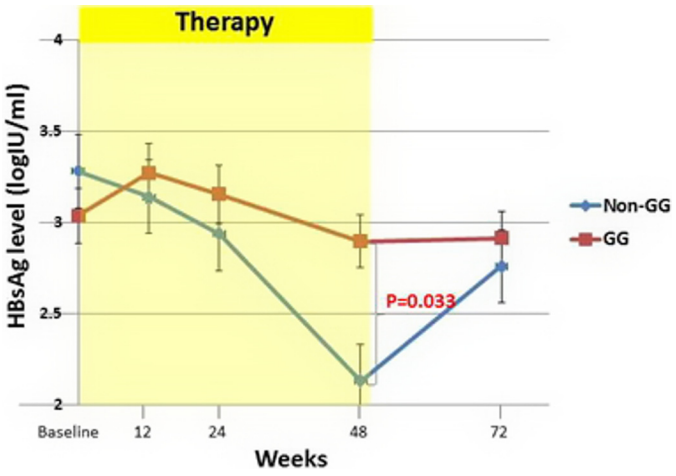

Figure 1.

P0613

HEPATITIS B CORE RELATED ANTIGEN MAY BE A MARKER FOR IMMUNE CONTROL IN HBeAg NEGATIVE CHRONIC HEPATITIS B INFECTION

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Background and Aims: Hepatitis B core related antigen (HBcrAg) is a new serum marker comprised of 3 viral proteins coded by the precore/core region of the covalently closed circular DNA (cccDNA): HBeAg, HBcAg and p22cr. Our aims were to describe HBcrAg kinetics in HBeAg negative chronic hepatitis B (CHB), and to assess if HBcrAg can be used as a marker for immune control in this group.
Methods: We measured serum HBcrAg in patients who participated in an international multicenter RCT (ARES study). In this study, 175 HBeAg positive patients all received entecavir (ETV) treatment for 24 weeks, and were then allocated to either continuation of ETV monotherapy or addition of 24 weeks of peginterferon alpha-2a (PEG-IFN add-on). Only when combined response (CR; HBeAg loss and HBV DNA <200 IU/mL) was achieved at week 48, patients stopped treatment at week 72. For this post-hoc analysis, we focused on HBcrAg decline between week 72 and 96 in patients who were HBeAg negative in this time interval. Also, viral relapse (HBV DNA >200 IU/mL) was assessed. HBcrAg levels were measured using the Lumipulse® G HBcrAg assay (Fujirebio Europe, Belgium).
Results: HBeAg was negative at both week 72 and 96 in 36 patients. In 16/36 patients ETV was stopped at week 72. In this group, HBcrAg levels declined in 10 patients, remained stable in 1 patient and increased in 4 patients (Figure 1). Week 72 HBcrAg was missing in 1 patient. In all 4 patients with HBcrAg increase, viral relapse occurred at week 96. In contrast, viral relapse was observed in only 1/10 patients with HBcrAg decline. In 20 patients who continued ETV between week 72 and 96, HBcrAg declined in 12 patients and increased in 8 patients. No viral relapse occurred in this group. One patient of the PEG-IFN add-on arm who had HBcrAg decline (−0.65 log U/mL) cleared HBsAg at week 96. Overall, mean HBcrAg decline was stronger in patients who previously received PEG-IFN add-on therapy than in patients of the ETV monotherapy arm (−0.29 vs. +0.14 U/mL, p = 0.033).
Conclusions: Serum HBcrAg levels declined in the majority of patients with PEG-IFN add-on induced HBeAg loss. HBcrAg increase was predominantly seen during ongoing ETV treatment of patients with ETV-induced HBeAg loss. Viral relapse after ETV cessation only occurred in patients who had HBcrAg increase or minor decrease. HBcrAg may therefore be useful as a marker for immune control in HBeAg negative CHB, but further analysis in larger patient groups is required.

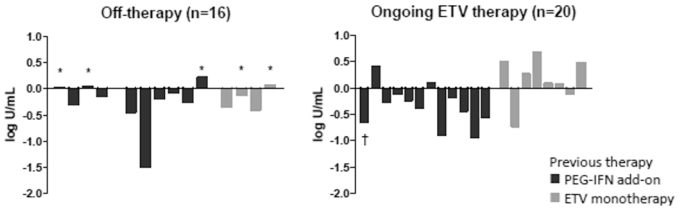

Figure 1. HBcrAg decline between week 72 and week 96. Bars represent individual log HBcrAg declines grouped by previous therapy. All patients were HBeAg negative between week 72 and 96 and had HBV DNA <200 IU/mL at week 72. Patients in the left panel stopped ETV at week 72, patients in the right panel continued ETV. *Viral relapse at week 96. † HBsAg loss at week 96.

P0614

KEY HBsAg MUTATIONS SIGNIFICANTLY CORRELATE WITH HCC, HAMPER HBsAg SECRETION AND PROMOTE CELL PROLIFERATION IN VITRO

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Background and Aims: To investigate HBsAg genetic determinants correlated with HBV-induced hepatocellular carcinoma (HCC) and their impact on cell proliferation.
Methods: This study includes 153 HBV chronically infected patients: 15 with HCC (60% D; 33% A, 7% E HBV genotype), and 138 asymptomatic patients as control (58% D, 31% A, 11% E). Mutations were defined according to the reference sequence of each specific HBV genotype. Association of HBsAg mutations with HCC was assessed by Fisher test.

HBsAg mutations were introduced into a 1.3× genome-length HBV genotype D. WT and mutated clones were transfected into Huh7 cells. Lysates and supernatants were harvested in triplicate daily until day 5 post-transfection, and HBsAg (representing 24 hours of accumulation) quantified by Alexxis assay. Mutations were also introduced into a pIRES II plasmid encoding HBsAg and GFP. Cellular cycle was analysed by flow cytometry (DNA propidium iodide-staining) on transfected GFP+ cells at day 7 post transfection (4 experiments in triplicate).

Results: Patients with or without HCC had median (IQR) log serum HBV-DNA of 4.1 (3.2–6.0) and 4.1 (3.1–6.0) UI/ml, respectively. Two HBsAg mutations significantly correlated with HCC: P203Q (27% [4/15] in HCC vs 2% [3/138] in non-HCC, p = 0.002); S210R (40% [6/15] in HCC vs 12% [17/138] in non-HCC, p = 0.012); P203Q+S210R (27% [4/15] in HCC vs 1.4% [2/138] in non-HCC, p = 0.001). Both P203Q and S210R reside in transmembrane C-terminal domain known to be important for HBsAg secretion.

In vitro, the presence of P203Q, S210R and P203Q+S210R reduced the ratio of secreted/intracellular HBsAg compared to wt at each time point. Indeed, this ratio varied as follows: at 3 days P203Q (1.734±0.2; p < 0.005), S210R (2.54±0.7), P203Q+S210R (1.83±0.1; p < 0.005) vs wt (2.99±0.2); at 4 days P203Q (2.22±0.2; p < 0.005); S210R (3.52±0.2; p < 0.05), P203Q+S210R (2.33±0.2; p < 0.01) vs wt (4.42±0.3), and at 5 days P203Q (1.15±0.1; p < 0.005), S210R (1.69±0.1; p < 0.005), P203Q+S210R (1.25±0.1; p < 0.005) vs wt (2.23±0.1).

By flow cytometry, P203Q and P203Q+S210R significantly correlated with an increased percentage of cells in the S phase, indicating cell cycle progression: P203Q (26±13%) and P203Q+S210R (29±14%) compared to wt (18%±9) (p ≤ 0.01).

Conclusions: Key mutations, residing in C-terminal HBsAg domain, are highly correlated with HBV-induced HCC *in vivo*. They affect HBsAg secretion and stimulate cell proliferation *in vitro*, suggesting their potential involvement in HCC development.

P0615

DIAGNOSIS BY SCREENING RATHER THAN SYMPTOMS IN HBV-RELATED HCC IS ASSOCIATED WITH IMPROVED OUTCOMES, REGARDLESS OF CIRRHOSIS STATUS BUT SCREENING HISTORY WAS ONLY PRESENT IN ONE-HALF OF CIRRHOSIS AND ONE-THIRD OF NONCIRRHOSIS CASES

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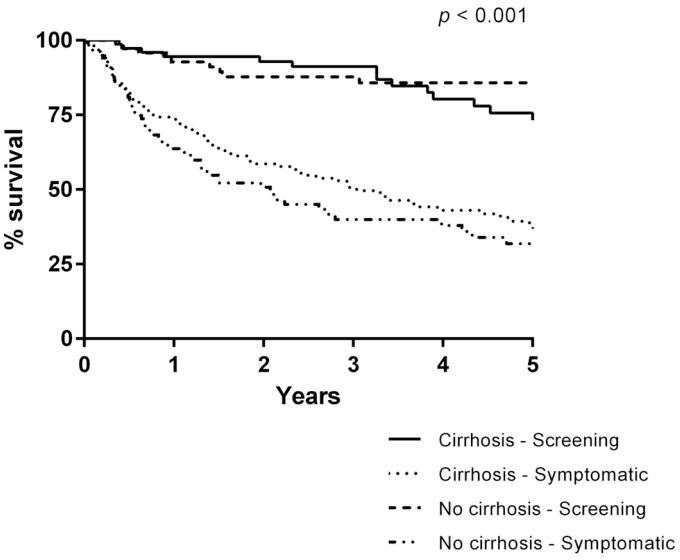
Background and Aims: Regular HCC surveillance is recommended by major hepatology societies in patients with chronic hepatitis B (CHB) including those without cirrhosis. However, adherence to surveillance is inconsistent in clinical practice. We investigated the impact of HCC screening on tumor characteristics and patient outcomes in patients with HBV-related HCC with and without cirrhosis.

Methods: We investigated HCC diagnosis scenario (by screening vs. by symptoms) in a cohort of 493 consecutive patients with HBV-related HCC as confirmed by AASLD criteria at a U.S. tertiary care center between 2005 and 2014. "Screening group" patients are those diagnosed by screening imaging in the absence of symptoms while "symptomatic group" patients are those diagnosed with symptoms of HCC. Survival was determined using chart review and/or National Death Index search.

Results: Mean age was 60; 80% were male; 89% were Asian; and 62% had cirrhosis. Patients without cirrhosis were more often diagnosed by symptoms than those with cirrhosis (45% vs. 36%; $p = 0.01$). Compared to those in the symptomatic group, a much greater proportion of screening group patients was eligible for liver transplantation by Milan criteria: 50.6% vs. 22.5% (cirrhosis) and 62.5% vs. 10.5% (no cirrhosis); $p < 0.001$. This difference appeared greater in patients without cirrhosis. Screening group patients were also much more likely to have BCLC stage A/B than symptomatic group patients, especially among those without cirrhosis: 92.6% vs. 59.7% (no cirrhosis) and 84.2% vs. 45.1% (cirrhosis); $p < 0.001$. A greater proportion of screening group patients underwent surgical treatment than did symptomatic group patients: 48.8% vs. 19.1% (cirrhosis) and 58.4% vs. 23.3% (no cirrhosis); $p < 0.001$. Finally, five-year survival was much greater in the screening than in the symptomatic group for patients with and without cirrhosis, though the difference appeared greater in those with cirrhosis (Fig. 1).

Conclusions: HCC diagnosis by routine screening rather than by symptoms is associated with less advanced disease at time of diagnosis, greater likelihood to undergo curative treatment, and improved survival in patients with CHB with and without cirrhosis. Unfortunately, HCC was diagnosed by screening in only half of the patients with cirrhosis and one-third of those without cirrhosis. Further physician and patient education is needed to improve HCC screening adherence in CHB patients, especially in those without cirrhosis.



P0616

DESIGNING ENHANCED PATHWAYS OF CARE FOR PATIENTS WITH CHRONIC HEPATITIS B USING HepBase, A PROPRIETARY DATABASE USED TO STRATIFY PATIENTS

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Background and Aims: Greater awareness of chronic hepatitis B (CHB), improved therapy and clinical guidelines have all increased patient referrals to liver clinics. Improved understanding of disease progression and fibrosis biomarkers has created the opportunity to redesign pathways of care.

This study aims to use proprietary database software and non-invasive tests to stratify patients attending the Royal Free London (RFL) Hospital for CHB to allow identification of patients who are at low risk of disease progression allowing exploration of alternative follow-up arrangements.

Methods: HepBase, (Sciensus Health Informatics, Brighton) is a real-time clinical and research database, which uses ODBC, SPSS and MATLAB computing programmes to analyse hospital electronic medical record system and pharmacy data to generate patient

cohorts. Patient care pathways were designed in consultation with clinical colleagues based upon EASL clinical care guidelines to identify inactive or pharmacologically controlled CHB who have either (a) mild fibrosis or (b) advanced fibrosis/cirrhosis.
Inclusion criteria included: e-antigen positive or negative, ALT3.25. Patients discharged, transferred, lost to follow up or died were excluded.
Results: Between January 2011 and October 2013 the records of 1796 patients were entered in the RFL HepBase. Of these 867 (48%) satisfied the study's inclusion criteria (Table 1).
Groups 1 and 2 (36%) are considered low risk for disease progression and will be reviewed for allocation to a nurse-led, consultant monitored follow up clinic. 27 patients (FIB4 >3.25) are at risk of advanced fibrosis/cirrhosis. 70% were already diagnosed as cirrhotic. An additional 22% were under close monitoring for advanced liver disease. 9 patients with FIB4 >3.25 were not on treatment with an active decision to withhold treatment documented in five cases.

Table 1.

Group No.	Total	Not on treatment	On treatment
Group 1: HbeAg+, FIB4 <1.45	56	13	43
Group 2: HbeAg−, FIB4 <1.45	584	450	134
Group 3: HbeAg+, FIB4 >3.25	3	0	3
Group 4: HbeAg−, FIB4 >3.25	24	9	15
Unclassified: FIB4 >1.45 and <3.25 or no platelet value	200	127	73
Total	867	602	265

Conclusions: Using an integrated database, 36% of CHB clinic attenders have been identified as inactive or pharmacologically controlled CHB who might be eligible for alternative pathways of care.
Use of HepBase has facilitated redesign of pathways of care for patients with CHB, identifying those who might benefit from less intensive hospital management and those who might benefit from closer monitoring for complications of advanced CHB and consideration of treatment. This approach may also reduce pressure on hospital clinics. We will go on to determine the effectiveness, cost-effectiveness and acceptability of these strategies.

P0617
IS HBeAg SEROCONVERSION A VALID SURROGATE MARKER FOR TRANSPLANT-FREE SURVIVAL IN CHRONIC HEPATITIS B PATIENTS TREATED WITH LAMIVUDINE OR ENTECAVIR?

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Background and Aims: A valid surrogate marker should be predictive of future true clinical outcome of interest to be used to assess the efficacy of disease-modifying interventions. HBeAg seroconversion has been identified as a valid surrogate marker of the survival during the natural course of patients with chronic hepatitis B (CHB). However, the validity of HBeAg seroconversion as a surrogate marker for transplant-free survival during nucleos(t)ide analogue treatment for CHB is not clear.
Methods: We performed a retrospective analysis of data from 5374 consecutive adult patients with CHB, treated with lamivudine (LAM, n = 3374) or entecavir (ETV, n = 2000) at a tertiary referral hospital in Seoul, Korea from 1999 through 2011. Data were analyzed by time-dependent multivariable Cox proportional hazards model.
Results: HBeAg was positive in 3587 (66.7%) patients at baseline; 2419 (71.8%) in the LAM group and 1168 (58.4%) in the ETV group. During the study period, 302 patients (5.6%) died, and 169 (3.1%) received a liver transplant. Multivariable analyses showed that baseline HBeAg-positivity was not an independent risk factor for lower risk of death or transplantation in the entire cohort (hazard ratio [HR], 0.92; 95% confidence interval [CI], 0.78–1.10). Among HBeAg-positive patients at baseline, 1425 (39.7%) achieved HBeAg seroconversion during treatment. In the LAM group, HBeAg seroconversion was significantly associated with a reduced risk of transplant or death (HR, 0.60; 95% CI, 0.48–0.77). On the contrary, HBeAg seroconversion was not associated with the risk of transplant or death in the ETV group (HR, 1.67; 95% CI, 0.78–3.60).
Conclusions: In a large cohort of CHB patients, HBeAg seroconversion was associated with a significantly lower risk of transplant or death during treatment with LAM. However, the significance of HBeAg seroconversion as a surrogate marker for transplant-free survival was not identified during treatment with a more potent antiviral agent, ETV.

P0618
ANTI-HEV IgG SEROPREVALENCE IN EUROPE: A META-ANALYSIS

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Background and Aims: Hepatitis E is of emerging relevance in Europe. Many early studies showed anti-HEV IgG seroprevalence rates <5%. However, HEV seems to be very common in Europe, as evidenced by high numbers of blood donors who are viraemic at the time of donation. Aim: to compare published anti-HEV IgG seroprevalence estimates in Europe.
Methods: Comprehensive literature review (Pubmed) of English-language anti-HEV IgG seroprevalence surveys in European countries between 01/2004 and 10/2014. Chi square test was used to compare seroprevalence rates.
Results: 82 studies and 1,602,429 individuals (1,592,845 healthy, 3752 HIV-infected, 2742 transplants, 3090 people with contact with swine/wild animals) were included. The most frequently used assays were Wantai (WT), MP-diagnostics (MP) and Mikrogen (MG). Observed seroprevalence rates in healthy populations showed huge differences (WT: 21.2%, MP: 6.5%, MG: 17.8%, WT vs MP, WT vs MG and MP vs MG, $p < 0.0001$ for each comparison). Comparing European nations, the highest seroprevalence rate was found in France (WT: 31.1%, MP: 18.7%) and the lowest in Spain (WT: 4.1%, MP: 3.5%, France vs Spain: WT $p < 0.0000$, MP $p < 0.0000$). Seroprevalence rates in patients with solid organ transplant recipients or HIV-infected patients were not different when compared to healthy controls (table), whereas individuals with contact with swine/wild animals (farmers, veterinarians, forestry workers) showed a significantly higher HEV seroprevalence (WT: $p < 0.0001$, MP: $p < 0.0001$, MG: $p = 0.0153$).
Conclusions: HEV seroprevalence varies considerably and is dependent on the assay employed, geographical location and patient group. None of the assays currently in use have been completely validated with respect to detection of distant infection. However, WT has a sensitivity of 98% in this regard, but its specificity is unknown [1]. Its specificity is probably acceptable, as in some populations seroprevalence estimates using this assay are very low (e.g. Fiji, 2% [2]). A comprehensive validation exercise of existing serology is needed, together with the establishment of WHO reference materials for HEV serology.

Reference(s)

[1] Bendall et al, J Med Virol 2010; 82: 799–805.
[2] Halliday et al. EID. 2014 Jun; 20(6): 1057–8.

Table (abstract O0618).

	Wantai	MP	Mikrogen	Dia.Pro	Adaltis	Others	Total
Healthy, seroprevalence (%)	21.2	6.5	17.8	12.4	0	10.8	21
Study cohort n =	1,560,047	16,815	8,962	3,345	0	2,707	1,592,845
HIV, seroprevalence (%)	22.9	3.7	nd	9	5.8	9	9.6
Study cohort n =	751	1,357	0	548	429	238	3,752
Transplant, seroprevalence (%)	24.1	6.5	2.9	13	10.9	nd	11.7
Study cohort n =	621	1,676	207	328	64	0	2,742
Swine/Foresty worker/veterinarians, seroprevalence (%)	48.8	29.7	22.1	nd	nd	50.3	30.1
Study cohort n =	43	2,253	503	0	0	291	3,090

P0619

ASSOCIATION OF THE GENE POLYMORPHISMS IN SODIUM TAUROCHOLATE COTRANSPORTING POLYPEPTIDE WITH HBV-RELATED HEPATIC FIBROSIS

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Background and Aims: Progressive hepatic fibrosis with the development of cirrhosis is a feature in the majority of chronic hepatitis B virus (HBV) infection cases. Sodium taurocholate cotransporting polypeptide (NTCP) was newly identified as a HBV receptor and required for efficient viral entry. The aim of the study was to investigate the potential relationship between hepatic fibrosis/cirrhosis and NTCP polymorphisms in HBV patients.
Methods: A total of 581 consecutive patients with chronic HBV infection were retrospectively included for this analysis. Liver fibrosis/cirrhosis was explored by calculating FIB-4 index, the cutoff value were 1.62 and 3.25 for significant fibrosis and cirrhosis, respectively. Three single nucleotide polymorphisms (SNPs) in NTCP were genotyped by high resolution melting (HRM) curve method.
Results: 327 (56.3%) and 150 (25.8%) patients were identified in the study as significant fibrosis and cirrhosis, respectively. Rs4646287 AA genotype was more common in significant fibrosis group (2.1% versus 0.0%, $p = 0.020$ in a recessive model). Furthermore, we found that A allele of rs4646287 was overrepresented in significant fibrosis group as well (11.2% versus 7.3%, $p = 0.025$). No significant differences in frequency distributions of the other two SNP (rs7154439 and rs4646296) alleles or genotypes between case and control groups were found in this study (all $p > 0.05$).
Conclusions: This study suggests that polymorphisms in the NTCP region may be associated with the hepatic fibrosis of HBV infection. The patients with rs4646287 AA genotype may be prone to HBV-related liver fibrosis. However, larger studies, most likely through multicenter collaboration will be needed to fully validate the significance of these findings.

P0620

IMPROVING REFERRAL OF WOMEN WITH CHRONIC HEPATITIS B FROM ANTENATAL SERVICES TO A SPECIALIST HEPATITIS CLINIC – THE NORTHERN IRELAND EXPERIENCE

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Background and Aims: Antenatal screening for hepatitis B in all pregnancies has been recommended in the UK since 1998. Previous Northern Ireland (NI) data showed that only a small proportion of these women were reviewed at a specialist hepatitis clinic[1]. In 2011, a new referral form and referral pathway were introduced to improve follow-up of all pregnant women with a positive HBsAg.
The aim was to re-audit the follow-up of pregnant women with a positive HBsAg in NI.
Methods: Data from NI maternity services, the Regional Virology Laboratory and the NI Hepatitis B&C Clinical Network were reviewed from 2004–2013 to identify all antenatal HBsAg positive patients. Data from 2004–2010 was analysed retrospectively. In 2011 a new referral pathway was introduced by the NI Hepatitis B&C Clinical Network, and data was gathered prospectively, including reason for non-attendance at specialist clinics. All women with an HBV DNA $>10^7$ were considered for oral antiviral treatment in the 3rd trimester.
Results: Table 1 shows the number of HBsAg positive women detected and referred each year. Up until 2010 the number referred to specialist hepatology services was <60%. Following introduction of the new pathway this figure increased to ≥94%.
Review of ethnicity data for 2013 showed that the vast majority of patients were of non-UK origin (19 South-East Asia, 13 Eastern Europeans, 5 Africans, 7 unrecorded and 1 other region). This was similar to the 2004–2010 data.
Conclusions: Intervention by the NI Hepatitis Network in 2011 dramatically improved the rate of referral of HBsAg positive women from maternity services to a specialist hepatitis clinic (from <60% to >90%).

Table 1. Antenatal referral and review of HBsAg positive patients in Northern Ireland

Year	Number of HBsAg positive cases detected	Number (%) of referrals to hepatology	Number (%) seen by hepatology	Patients requiring oral antivirals
2004	25	2 (8%)	N/A	–
2005	36	7 (19%)	N/A	–
2006	29	10 (34%)	N/A	–
2007	46	22 (48%)	N/A	–
2008	39	18 (46%)	N/A	–
2009	36	21 (58%)	N/A	–
2010	45	22 (49%)	N/A	–
2011	35	33 (94%)	27 (82%)	1
2012	37	35 (95%)	31 (89%)	2
2013	45	43 (96%)	36 (84%)	2

Reference(s)

[1] Detection of chronic hepatitis B through antenatal screening in NI: what happens to the mother? E. Mamwa et al. ISG oral presentation 2011.

P0621

HEPATITIS E VIRUS IN PATIENTS WITH DECOMPENSATED CHRONIC LIVER DISEASE AND ALCOHOLIC HEPATITIS: A UK/FRENCH STUDY

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Background and Aims: In developed countries hepatitis E is a porcine zoonosis caused by HEV genotype 3. In developing countries hepatitis E is mainly caused by genotype 1, and causes increased mortality in patients with pre-existing chronic liver disease which may approach 70%. Aim: determine the role of HEV in patients with decompensated chronic liver disease and/or alcoholic hepatitis.
Methods: Prospective HEV testing of 372 patients with decompensated chronic liver disease and/or alcoholic hepatitis at three UK centres and Toulouse France, with follow-up for 6 months or death. In addition, IgG seroprevalence was compared with 911 controls.
Results: 11/372 patients (2.9%) had acute hepatitis E infection, three died. There were no differences in mortality (27% vs 26%, OR1.1, 95% CI 0.28–4.2), mode of clinical presentation (p = 0.4), bilirubin (p = 0.5), ALT (p = 0.06) albumin (p = 0.5) and INR (p = 0.5) in patients with and without hepatitis E infection. Five cases were PCR positive (genotype 3). Hepatitis E was more common in Toulouse (7.9%) compared to the UK cohort (1.1%, p < 0.001). HEV IgG seroprevalence was higher in Toulouse (OR 17, 95% CI 9.6 to 31) and Truro (OR 2.6, 95% CI 1.4 to 4.7) than in Glasgow, but lower in cases, compared to controls (OR 0.59, 95% CI 0.41 to 0.85).

Case	1	2	3	4	5	6	7	8	9	10	11
Centre	Truro	Truro	Norwich	Toulouse	Toulouse	Toulouse	Toulouse	Toulouse	Toulouse	Toulouse	Toulouse
Age (years)	60	54	60	56	50	59	53	54	75	59	61
Sex	M	M	M	F	M	M	M	M	M	M	M
HEV IgG/IgM	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/+	+/+	+/+	-/+
HEV PCR	+	-	-	-	+	NA	+	-	+	-	+
Genotype	3				3		3		3		3
Bilirubin (µmol/L)	304	22	45	275	173	61	292	151	797	42	451
ALT (IU/L)	2419	10	70	129	128	129	2060	30	215	126	95
INR	1.5	0.9	1.6	2.1	NA	1.7	NA	1.6	2.3	1.2	NA
Diagnosis	dCLD	dCLD	dCLD	dCLD & AH	dCLD	dCLD	dCLD	dCLD	dCLD	dCLD	dCLD
Cause of liver disease	NASH	Alcohol	Alcohol	Alcohol	Alcohol HBV	HCV NASH	Alcohol	Alcohol	Alcohol	NASH	Alcohol
Child-Pugh	8	5	NA	11	10	10	11	8	14	10	13
Death <3 months	No	No	No	No	No	No	No	Yes	Yes	No	Yes

Conclusions: Hepatitis E occurs in a minority of patients with decompensated CLD and/or alcoholic hepatitis. The mortality is no different to the mortality in patients without hepatitis E infection. The diagnosis can only be established by a combination of serology and PCR, the yield and utility of which vary by geographical location. An early diagnosis is important as patients may respond favourably to anti-viral therapy.

P0622

EFFECTIVENESS OF SEVERAL SIMPLE AND NONINVASIVE MODELS IN ASSESSING LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B

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Background and Aims: The present study was conducted to investigate the influence of hepatic inflammation histologically on liver stiffness measurement (LSM) measured by FibroScan in diagnoses of liver fibrosis.
Methods: Three hundred and twenty-five patients with chronic hepatitis B (CHB), who received liver biopsy and FibroScan, were included. According to Scheuer scoring system, liver fibrosis and inflammation were divided into five stages (S0–S4) and grades (G0–G4).
Results: LSM was correlated positively with fibrosis stage and inflammatory grade (r = 0.479, P < 0.001; r = 0.522, P < 0.001, respectively). Though median value of LSM in the same fibrosis stage increased along with inflammatory grade, no significant difference was found in patients with significant fibrosis (S2–S4) (P > 0.05). In different grades of inflammation (G0/G1/G2/G3), the area under receiver operating characteristic curves of FibroScan in diagnosing significant fibrosis was 0.8267 (P < 0.001), 0.6956 (P < 0.001), 0.709 (P = 0.0012) and 0.6947 (P = 0.137), respectively.
Conclusions: LSM increased as the inflammatory grades increased, but no significant influence of inflammation on LSM in CHB patients with significant fibrosis. As for the diagnoses of significant fibrosis, FibroScan was not suitable for patients with severe hepatic inflammation.

P0623

A COMBINATION OF HEPATOCYTE DEATH BIOMARKER WITH THE MELD SCORE IMPROVES PREDICTIVE ACCURACY OF PROGNOSIS IN PATIENTS WITH HEPATITIS B VIRUS RELATED ACUTE-ON-CHRONIC LIVER FAILURE

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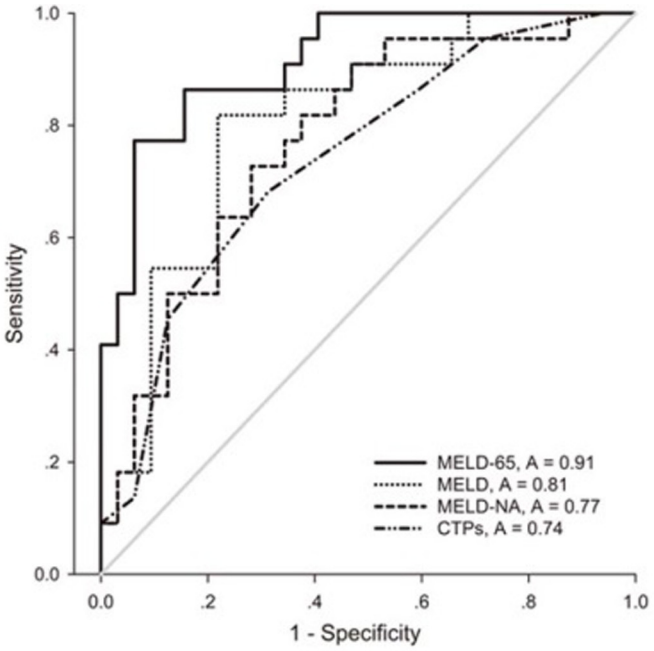
Background and Aims: Survival data from Asia-Pacific on Hepatiis B virus related acute-on-chronic liver failure (HBV-ACLF) patients are urgently needed to develop novel biomarkers or scoring systems to help determining when treatments such as intensive care alone, artificial liver support, or liver transplantation are optimal. Given the increasingly important roles for apoptotic and necrotic cell death in the pathogenesis of HBV-ACLF, we aimed to identify three selected potential hepatocyte death biomarkers that could be of prognostic value in HBV-ACLF patients.
Methods: In a prospective study (3/2013–8/2014), 54 ALF patients were enrolled and followed up for 3 months to identify clinical outcomes. 40 chronic hepatitis B patients and 40 healthy volunteers were recruited as controls. Serum were collected and stored upon admission of 54 ACLF patients or at the enrollment day of controls for subsequent measurements of M30-antigen, M65-antigen and HMGB1. Receiver operating characteristic (ROC) analyses were performed to compare the discrimination abilities of cell death biomarkers, MELD, MELD-sodium (MELD-NA), and Child–Pugh (CP) scores.
Results: M30-antigen, M65-antigen and HMGB1 markedly (P > 0.001 for all) elevated in HBV-ACLF patients compared with controls. The predictive accuracy of M30 (AUC: 0.86) and M65-antigen (AUC: 0.83) for lethal outcome was comparable to the

MELD score (AUC: 0.81) and better than MELD-NA (AUC: 0.78) and CP (AUC: 0.74) scores. In contrast, HMGB1 had no prognostic value (p = 0.681), neither in cirrhotic nor in non-cirrhotic HBV-ACLF patients. In order to accurately predict the clinical prognosis of HBV-ACLF patients, we test the MELD-65 index [MELD-65 = 0.000372×M65-antigen (U/L) + 0.3432×MELD score − 11.17] established by logistic regression analysis with MELD and M65-antigen remaining in the model. The new M65-based MELD score (AUC: 0.92, 95% CI 0.81 to 0.98, youden index: 0.71) provided significantly better predictive values in HBV-ACLF than MELD (AUC: 0.81, youden index: 0.6, P value vs MELD-65, P = 0.03), MELD-NA (AUC: 0.77, youden index: 0.45, P value vs MELD-65, P = 0.01) and CP (AUC: 74, youden index: 0.37, P value vs MELD-65, P = 0.02) scores.

Conclusions: The MELD-65 index, a combination of MELD score and M65-antigen, has superior predictive accuracy to MELD, MELD-NA and CTP scores for the prognosis of HBV-ACLF patients from Asia-pacific. Further prospective clinical studies are warranted to validate its role in predicting ACLF patients with other etiologies.



		AUROC (95% CI)	*p* value vs. MELD-65
———	MELD-65	0.91(0.84-0.99)	
·····	MELD	0.81(0.69-0.93)	0.03
- - - - -	MELD-Na	0.77(0.65-0.90)	0.01
—·—·—	CTP	0.74(0.61-0.87)	0.02

P0624

ACUTE VIRAL HEPATITIS: EPIDEMIOLOGICAL CHANGE DURING THE LAST 25 YEARS IN SPAIN

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Background and Aims: The childhood vaccination programs against hepatitis B virus (HBV) and hepatitis A (HAV) and the improvement in diagnosis of hepatitis E virus (HEV) may have changed the epidemiology of acute hepatitis in Spain. The goal of the present work is to analyze the aetiology of acute hepatitis, and to compare the epidemiologic change with an historic cohort from the same tertiary hospital.

Methods: Patients aged over 18 years old with increased ALT levels (10×ULN) admitted to a universitary hospital within October 2013 to November 2014 were prospectively included. Serological and virological tests (IgM antiHBc, HBsAg, antiHCV, HCV RNA, IgM HEV, IgM HAV) were performed in all patients without biliary obstruction or shock. Acute viral hepatitis cases were contrasted to an historic cohort from 1990 collected in the same hospital [Buti M, *et al.* J Hepatol. 1994].

Results: 124 patients were included. 54% were male, median age 46 years old [IQR 34–65]. 32 (25.8%) were diagnosed with viral hepatitis. The rest of the aetiologies were as follow: biliary obstruction (37.1%), ischemic (13.7%), toxic (10.5%), autoimmune hepatitis (9.7%) and alcoholic (3.2%). Viral hepatitis was caused by HBV (37.5%), HCV (21.9%), HEV (15.6%), EBV (9.4%), HDV (3%), HAV (3%), influenza (3%), measles (3%) and VZV (3%). In the current cohort there were 5 autochthonous cases of hepatitis E in contrast to none in the historic one, and genotype 3 was isolated in those patients with high levels of RNA HEV. In comparison with the cohort from 1990, an increase in the percentage of acute hepatitis due to HBV was observed (18%), in spite of systematic vaccination during childhood since 1992, so outside the range of age that have received HBV vaccination (Figure). There was only one acute hepatitis A, from a traveler from Morocco. 59% of acute viral hepatitis cases were admitted to hospital. 19% developed acute liver injury and 12.5% of cases died.

Conclusions: Acute viral hepatitis remains a frequent cause of admission to hospital, with a mortality rate around 12%. In contrast to 1990, there have been cases due to HEV likely due to the improvement in diagnostic tests, and also an increase in the cases due to HBV, all of them in patients older than 23 years old. This data suggests that patients older than 23 years old may benefit from HBV vaccination.

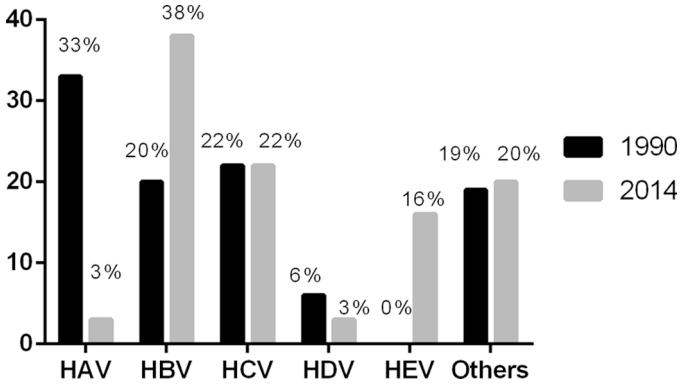

Figure: Comparison of the main causes of acute viral hepatitis.

P0625

A HYPER-GLYCOSYLATION OF HBV SURFACE MAJOR HYDROPHILIC REGION CHARACTERIZES HBV REACTIVATION DRIVEN BY IMMUNOSUPPRESSION AND AFFECTS HBsAg RECOGNITION IN VITRO

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Background and Aims: To investigate N-linked glycosylation patterns of HBsAg in immunosuppression-driven HBV-reactivation and to evaluate their impact on HBsAg antigenicity.
Methods: Mutations associated with the acquisition of N-glycosylation site were investigated in 127 HBsAg genotype-D sequences from 47 patients with immunosuppression-driven HBV-reactivation (defined as Hwang, 2013), and 80 chronically HBV-infected drug-naïve patients as control.
The impact of N-glycosylation sites on HBsAg antigenicity was analyzed by transfecting HepG2 cells with a plasmid encoding wild-type (wt) and mutated HBsAg-linked to a streptavidin-tag (strep-tag). The strep-tagged HBsAg amount in the supernatants was quantified by a specifically-designed ELISA recognizing the Strep region (thus, not affected by HBsAg mutations), and by two ELISAs directly targeting the HBsAg (Architect Abbott, Monolisa Biorad).
Results: Additional N-glycosylation sites are found in 19.1% (9/47) of HBV-reactivated patients versus 0/80 controls ($P < 0.001$). All of them localize in the major hydrophilic HBsAg region, target of antibodies. In 7 patients, a single additional N-glycosylation site results from the mutations T115N ($n = 2$), T123N ($n = 2$), T131N ($n = 2$), and from the insertion of an N between 114 and 115 position (ins114–115N) ($n = 1$). In the remaining 2 patients, 2 additional glycosylation sites results from the mutations S113N+T131N and ins114–115N+T117N, respectively.
Of note, 5/9 patients with ≥1 additional N-glycosylation sites result HBsAg-negative by diagnostic test at HBV-reactivation ($p = 0.002$).
In vitro, all additional N-glycosylation sites decrease the strep-tagged HBsAg quantification by the 2 ELISAs targeting the HBsAg. In particular, T115N, T123N, ins114–115N determine a >90% decrease in HBsAg quantification by both ELISAs. Similarly, ins114–115N+T117N cause a 90.2% and 75.4% reduction in HBsAg quantification, respectively. No decrease of strep-tagged HBsAg is revealed by ELISA targeting the Strep-tag. Overall results suggest that the additional N-glycosylation sites hamper HBsAg recognition by antibodies without affecting HBsAg release.
Conclusions: Additional N-glycosylation sites in the major hydrophilic HBsAg region correlate with false HBsAg negativity at ELISA despite HBV reactivation, and profoundly affect HBsAg antigenicity *in vitro*. This supports the role of immune escape mutations in HBV reactivation during immune-suppression and the importance of HBV-DNA (more than HBsAg) in the diagnosis of HBV reactivation

P0626

THE ANALYSIS OF THE EXPRESSION OF A2M, COL1A1, MMP2 AND CHI3L IDENTIFIES SIGNIFICANT FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B

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Background and Aims: Chronic hepatitis B (CHB) remains a major health problem affecting approximately 350 millions people worldwide. Staging liver fibrosis is crucial in patients with CHB because it reflects the progression of the disease. The aim of the study was to identify differential liver gene expression between no and mild fibrosis (Metavir F0–F1) and moderate, severe fibrosis and cirrhosis (F2–F4), in a large cohort of unselected and treatment-naïve patients with CHB.
Methods: The expression of 102 genes was assessed in liver biopsies of 46 patients with CHB. Twenty-nine patients were HbeAg positive. Fibrosis was staged according to the Metavir score system (F0 to F4); 19 patients had no or mild fibrosis (F0 and F1) and 27 had moderate, severe fibrosis or cirrhosis (F2, F3 or F4). Genes with significant difference of expression, were assessed in liver biopsies of 144 patients with CHB (72 F0–F1 and 72 F2–F4), in an independent set of experiment. In this group, 100 patients were HbeAg positive. Gene expression was assessed by RT-qPCR.
Results: From the 102 genes studied, in the first group of 46 patients, 35 and 7 were respectively up-regulated and down-regulated in patients with F2–F4 compared to patients with F0–F1 ($p < 0.05$). In the second set of experiment, from the 42 genes studied, 16 were differentially expressed between F0–F1 and F2–4 ($p < 0.05$) (A2M, CCL4, CDKN1A, CHI3L1, CLDN4, CLL21, COL1A1, COL4A1, SCXL10, IL8, ILB, ITGAV, MMP2, MMP7, TGFB and VIM) In multivariate analysis, A2M ($p < 0.0001$), COL1A1 ($p = 0.060$), MMP2 ($p = 0.0141$) and CHI3L1 ($p = 0.0141$) were all up-regulated in patients with F2–F4 as compared to those with F0–F1. The model combining A2M, COL1A1, MMP2 and CHI3L1 discriminates patients with F0–F1 from those with F2–F4 with and AUC of 0.85 (95% CI: 0.79–0.91), a sensitivity of 70.5% a specificity of 80.8%, a positive predictive value of 79.7% and a negative predictive value of 72.0%. Interestingly, with an OR <1, the over-expression of MMP2 protects against significant fibrosis (F2F4).
Conclusions: We identified a model including 3 genes (A2M, COL1A1 and MMP2) known to play important roles during fibrosis. In patients with CHB, the assessment of A2M, COL1A1, MMP2 and CHI3L1 may help to distinguish patients with F0–F1 from those with F2–F4.

P0627

MONOCLONAL GAMMOPATHY IN PATIENTS WITH ACUTE HEPATITIS E AND OTHER CAUSES OF ACUTE VIRAL HEPATITIS

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Background and Aims: Hepatitis E virus (HEV) may be associated with serum monoclonal paraprotein (M-protein) production. It is unknown if M-protein is found in other causes of acute hepatitis.
Methods: We retrospectively compared M-protein prevalence at diagnosis in 46 patients with acute HEV and 54 cases of acute viral hepatitis caused by other agents (hepatitis A, B, C, CMV and EBV)

from 1054 consecutive patients presenting to a fast-track jaundice clinic over a 16 year period.
Results: M-protein was found in 10/39 (25.6%) of patients with HEV, but 0/37 (0%) of other causes of viral hepatitis (p = 0.001). M-proteins were IgG and/or IgM with a median concentration of 2.63 g/L (range from too small for accurate quantitation to 10.6 g/L). Follow up (n = 6, median 42.5 months) showed M-protein disappearance (n = 4), too small for accurate quantification (n = 1) or persistence at 54 months (n = 1). Compared to the comparator group, patients with HEV were older (p < 0.01). None were immunocompromised and all demonstrated HEV genotype 3 (when sequencing data was available) with close sequence homology (figure).
Conclusions: M-protein is commonly associated with acute hepatitis E infection, but not with other causes of viral hepatitis. The clinical significance of this observation remains uncertain and merits further study, particularly with regard to the risk of future lymphoid malignancy.

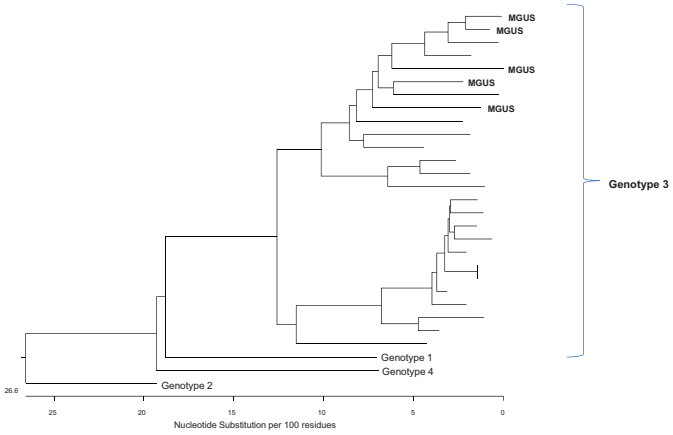

Figure: Phylogenetic tree of sequenced HEV in the patient cohort.

P0628
A HIGH GENETIC HETEROGENEITY IN HBsAg CAN AFFECT IMMUNOGENICITY IN ACUTE HEPATITIS B INFECTION
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Background and Aims: To characterize HBV RT and HBsAg quasispecies heterogeneity in acute HBV (AHB) infected patients and to define their clinical value.
Methods: 62 HBsAg+ and IgM/anti-HBc+ patients with clinical and biochemical signs of AHB (44 HBV genotype [gen] D and 18 gen A) were enrolled from 2000 to 2010. Plasma samples obtained at first observation were analyzed by Ultra-deep sequencing (UDPS) for drug-resistance (DR) and immune-escape mutations. Shannon Entropy, weighted for intra-patient prevalence of each mutated position (Sn), was used to measure the extent of HBsAg amino acid (aa) variability.
Results: 75.8% of patients were male with median (IQR) age of 36 (29–46) years. Median (IQR) ALT and HBV-DNA were 2,544 (1,938–3,078) U/L and 5.9 (4.5–7.4) log10 IU/ml. 61/62 (98.4%) patients became HBsAg negative with 33/61 (54.1%) developing also anti-HBs.
By UDPS, 8.1% (5/62) of patients carried >1 DR mutation (rtV173L/rtL180M/rtA181T/rtA194T/rtM204I). In particular, A181T was detected with an intra-patient prevalence of 0.1% and 47.5%, while both A194T and M204I were found at a prevalence of 0.2%. Compensatory mutations were detected with an intra-patient prevalence of 10.5% and 63.5% for V173L and, 99.9% for L180M.
Analysing HBsAg a-determinant, 48.4% (30/62) of patients carried /1 immune-escape mutation (intra-patient prevalence range: 0.2–100%). Vaccine-escape mutations were found in 11.4% of patients, all gen D-infected. This is the case of sG145R, sM133L, and sP120S, detected with an intra-patient prevalence ranging from 3.9% to 99.9% for sG145R, from 1.9% to 16.8% for sM133L, and always of 100% for sP120S.
Stop-codons were found in 19.3% patients (intra-patient prevalence range: 1.6–47.5%). They occurred at 11 HBsAg positions including also 172 and 182, known to increase HBV oncogenic potential.
Finally, by Shannon Entropy, specific HBsAg aa positions were associated with the lack of HBsAg seroconversion in gen D. In particular, aa positions 130 and 133 (localized in HLA class II epitope) were found mutated only in patients not developing anti-HBs (Sn, mean±SE:1.98±0.01 vs 0, and 1.95±0.03 vs 0, respectively, P < 0.05).
Conclusions: AHB is characterized by a complex coexistence of viral quasispecies, some of them with reduced antigenicity/immunogenicity, enhanced oncogenic-potential and altered drug-susceptibility. These viral variants may induce severe and/or difficult-to-treat forms of HBV infection (es. HBV reactivation), and might affect the efficacy of current HBV vaccination strategy.

P0629
FIRST DETECTION OF GENOTYPE 3 AND 4 HEPATITIS E VIRUS (HEV) RNA AMONG THE GENERAL POPULATION IN CAMBODIA: FULL-LENGTH SEQUENCE OF HEV ISOLATE, PREVALENCE AND INCIDENCE OF HEV INFECTION WERE CLARIFIED BASED ON THE SERO-EPIDEMIOLOGICAL STUDY
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Background and Aims: In Cambodia, one of the developing countries in Asia, hepatitis E virus (HEV) infection might remain an important health problem. However, few data are known about that in the country. Therefore, we investigated the HEV infection on seroprevalence, incidence, and genotypic distribution among the general population in Siem Reap province. Based on the results, we aim to consider the prevention strategy in cooperation with Ministry of Health in Cambodia.
Methods: This study was approved by the Ethics Committee for epidemiological research of Hiroshima University in Japan and that of Ministry of Health in Cambodia. We conducted the survey after obtaining written informed consents from all participants. Sero-epidemiological study, which consisted of questionnaires and taking blood samples for determining hepatitis virus infections, was performed from 2010 to 2014 among the general population in Siem Reap province. New HEV infection among the elementary school students was diagnosed who turned positive for IgG HEV antibody (anti-HEV IgG) during the observation period.
Results: For the prevalence, total participants were 857, comprising 355 males (41.4%) and 502 females (59.8%). Age distribution was from 7 to 90 years old as of 2013. Mean age was 30.7±18.7 years old. The prevalence of anti-HEV IgG was 18.7% (160/857; [95% CI: 16.1–21.3%]) and significantly higher in males (22.3% [17.9–26.6%]) than in females (16.1% [12.9–19.4%]) ($p = 0.0236$). Moreover, it showed the

trend that the older the age, the higher the positive rate ($p < 0.0001$). HEV RNA was detected in 2 (0.23% [0–0.56%]) participants. One of them could be sequenced over the entire genome and classified as genotype 4. This isolate was close to the swine China strain (swGX40) with 95.6% identity, and had the same length of ORF1, ORF2, and ORF3 as swGX40 and coded for 1,705, 674, and 114 amino acids, respectively. Another HEV RNA positive sample was classified as genotype 3 from a partial ORF1 genome sequence. New HEV infection developed in 1 of the 54 subjects (19 males and 35 females) who participated in our survey twice. The incidence of HEV infection was calculated to be 6.43/1,000 person-years [0.16–35.8/1,000 person-years].
Conclusions: Our study showed that the new HEV infection occurred frequently among the general population in Cambodia. This is the first report that HEV genotype 3 and 4 detected from people living in Cambodia.

P0630
VIROLOGIC CHARACTERIZATION AND ASSOCIATION WITH DISEASE PROGRESSION IN CHILDREN WITH HEPATITIS B VIRUS BCP/PC MUTANTS

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Background and Aims: To investigate the virologic characterization and association with disease progression in children with hepatitis B virus BCP/PC mutants.
Methods: A total of 307 patients with HBV infection, including 88 with hepatitis B related liver cirrhosis and 219 with CHB were enrolled. The HBV genotypes and the oresence of mutations in the BCP/PC regions were determined by direct sequencing. Biochemical and serological parameters as well as HBV DNA levels were routinely performed. Mutations at 11 interested sites of the BCP/PC regions were compared among the two groups of patients.
Results: 46/307 (14.98%) were infected with genotype B and 261/307 (85.02%) with genotype C. LC and CHB patients both had a significantly higher ratio of genotype C to B (81.9% > 18.1% vs 70.1% > 29.9%). The prevalence of BCP/PC wild-type virus was 54.3% in CHB patients in contrast to 4.8% in LC patients. In genotype C patients, the C1653T, T1753C, A1762T, G1764A, G1896A mutations were significantly higher prevalent in LC patients. Genotype B virus had higher 1752 mutation frequency. Genotype C virus had higher prevalence of T1753C, T1758C, A1762T, G1764A, G1896A mutation frequency compared to genotype B virus. CHB patients with BCP/PC mutant virus had higher viral load, whereas LC patients with BCP/PC mutant virus had higher viral load and elevated alanine aminotransferase in comparison with those with the wild type virus.
Conclusions: Children patients with HBV genotype C virus infection, BCP/PC C1653T, A1762T/G1764A, G1896A mutant were more susceptible to develop LC, whereas high prevalence of the BCP/PC mutations was associated with CHB progression.

P0631
THE HEPATITIS E VIRUS GENOTYPE 2 JUNCTION VARIANT RESTORES THE CREX 'STEM-LOOP' STRUCTURAL INTEGRITY, ESSENTIAL FOR VIRAL LIFE CYCLE

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Background and Aims: Of the human hepatitis E virus (HEV) strains (genotypes 1–4), the Mexican (genotype 2) isolate has two 'double-base' substitutions (5′U5100G5101→CU/3′C5117U5118→GG) flanking the conserved cis-reactive element (CRE) in the ORF1–ORF3 junction sequences. While 'CU' mutations in the 5′ ORF1 coding-region replaces 'Ala' for 'Val', 'GG' resides in the 3′ ORF3 promoter-region. In this study therefore, the evolutionary significance of such tolerable mutations in the conserved sequences was investigated.
Methods: GeneBank sequences (n = 77) of human HEV junction-region (nt. 5099–5121) including CRE and flanking bases (named, CREX) were analyzed in silico. Study sequences of SAR55, Mexican, Swine and T1 strains representing genotypes 1–4, were subjected to RNA structural analysis. Virtual mutations (μ1-U5118G and μ2-C5117A/U5118G) were introduced to break the 'C:G' and 'U:G' parings in the CREX 'stem-loop'. CREX mutants were constructed in SAR55 replicon (pSK-GFP) and tested in S10-3 cells. Further, trans-encapsidation of mutant-RNA in baculovirus-expressed capsid proteins were performed, followed by virion infectivity assay in naïve HepG2 cells. ORF2 productions in S10-cells were confirmed by immune-staining, and GFP +ve cells were scored by FACS to quantify the replication and packaging fitness of mutant RNA.
Results: Analysis of the conserved sequences (nts. 5099–5121) of the four genotypes revealed a stable CREX 'stem-loop' structure. Of these, the Mexican mutant bases 'CU/UG' very interestingly, compensated and complemented themselves (5′C5100:3′G5118 and 5′U5101:3′G5117) in the CREX 'lower-stem'. Substitution of 'GG' in the ORF3 promoter-region however, did not affect its 'optimal-context'. Virtual mutations introduced to break the two base-pairings in the CREX 'lower-stem', completely destabilized the secondary structure. At day 6, FACS analysis scored a remarkable reduction in the GFP +ve cells harboring CREX-μ1 (~62%) and CREX-μ2 (~75%) RNA. Further, the two mutant-virions produced in S10-3 cells when infected to naïve cells, showed a remarkable 3- and 6-fold reduction in GFP production, respectively. While the infectivity of the mutant virions were comparable they were poorly infectious (~33%) compared to the wild-type particles.
Conclusions: The compensatory mutations in the intergenic-junction of Mexican isolate suggest strict conservation of the CREX 'stem-loop' structure, essential for HEV genome replication. This could have a greater regulatory role in viral life cycle, including RNA packaging.

Viral hepatitis: Hepatitis B & D – c. Clinical (therapy, new compounds, resistance)

P0632
BISPECIFIC ANTIBODY CONSTRUCTS MEDIATE SPECIFIC RETARGETING TOWARDS AND ELIMINATION OF HBV-POSITIVE HEPATOCYTES AND TUMOR CELLS

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Background and Aims: Chronic hepatitis B is characterized by exhausted effector cells, incapable of eradicating the virus. To circumvent this limitation, HBV-specific retargeting of immune effector cells using bispecific monoclonal antibody (biMab) constructs is a promising therapeutic approach. In this study, effector cells are supplied with biMab that redirect T cells cells towards HBV infected cells and activate their cytotoxic potential.
Methods: We constructed tetravalent biMab harboring two single chain fragment (scFv) binding domains. The first scFv was designed to target the S-domain of HBV-envelope proteins (HBs) on the

plasma membrane of infected hepatocytes. The second binding motif engages T-cells via CD3 and co-stimulates them via CD28. The two binding moieties are connected through an IgG1-derived Fc-domain and dimerizes through disulfide bonding in the hinge region. This results in tetravalent homodimers comprising two scFv-binders for the target antigen and two for effector cell recruitment.
Results: In co-culture experiments, the engagement of HBV-positive hepatocytes and immune effector cells by the biMab induced specific elimination of target cells and activation of effector cells. Co-administration of HBs/CD3- and HBs/CD28-specific biMab constructs was synergistic and mediated up to 96% specific elimination of HBs-positive target cells in co-cultures with PBMC. Flow cytometric analysis of cytokine production showed, that retargeted effector cells were polyfunctionally activated by the biMab and secreted IFNγ, IL-2 and TNFα simultaneously. Importantly, biMab were also able to redirect T cells to HBV-infected HepaRG cell resulting in specific killing. Furthermore, soluble HBs, mimicking the high viral loads in patient serum failed to induce effector cell activation even when added to non-infected cells. Finally, first *in vivo* experiments in immunodeficient mice transplanted with HBV-positive hepatoma cells showed that transferred human PBMC mediated a marked decrease in tumor size upon biMab injection indicating a functional homing in tumors.
Conclusions: Retargeting of T cells towards HBV-positive cells using bispecific antibody constructs is a promising new immunotherapeutic approach to treat chronic hepatitis B and associated hepatocellular carcinoma.

P0633
ASSOCIATION OF HEPATITIS B VIRUS GENETIC DIVERSITY WITH TENOFOVIR DISOPROXIL FUMARATE RESPONSE RATES IN IMMUNE TOLERANT CHRONIC HEPATITIS B PATIENTS

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Background and Aims: Tenofovir disoproxil fumarate (TDF) is a nucleotide analogue reverse transcriptase (RT) inhibitor for the treatment of hepatitis B virus (HBV)-infected patients. TDF resistance has not been reported; however, varying viral suppression rates are observed among TDF-treated patients. We examined the association of HBV genetic diversity with response to TDF treatment in immune tolerant patients.
Methods: Study GS-US-203-0101 evaluated TDF in immune tolerant patients. 10 patients with genotypes (GT) B (n = 3), C (n = 6) and E (n = 1) were evaluated at baseline, week 4 and week 40. Five patients had a slow response (SR), never achieving HBV DNA <400 copies/mL, while 5 had a rapid response (RR), achieving HBV DNA <400 copies/mL by week 96. Patients were matched by baseline HBV DNA and ALT. Whole-genome sequences of HBV quasispecies (QS) were obtained using end-point limiting-dilution real-time PCR coupled with sequencing. Genetic diversity was evaluated using phylogenetic trees and median joining networks. Associations between amino acid (aa) or nucleotide (nt) substitutions and SR/RR to TDF treatment was examined with feature selection protocols, Bayesian Networks (BN), Artificial Neural Networks, Linear Projection techniques and multidimensional scaling.
Results: 1288 whole-genome QS were sequenced. Significant changes in the on-treatment intra-host population structure compared to baseline were observed in SR but not RR patients, suggesting differential TDF sensitivity of HBV QS. No association was found between TDF response and HBV RT substitutions. Of the 2033 substitutions evaluated, two nt substitutions in GT C RNase H (nt 1223 and 1322) were correlated among the 6 GT C patients ($R = 1.0$, $p = 0.1$) with SR/RR; this correlation was not observed for GT B or E. BN analysis revealed tight coevolution among HBV nt substitutions, with 16 nt across the Core, X, P, and S genes associated with SR/RR. Using these positions, the BN model classified HBV variants from these 10 patients into SR and RR classes with 99.7% accuracy (95% CI 99.4–100) in leave-one-out cross-validation tests.
Conclusions: We have studied a potential prediction model based on 16 nt substitutions in 10 patients across the HBV genome for response to TDF treatment. The contribution of nt substitutions to TDF response varied among HBV genotypes and was not localized to RT but involved the entire HBV genome. A larger patient set needs to be evaluated to confirm these findings.

P0634
PEGINTERFERON IS SUPERIOR TO NUCLEOS(T)IDE ANALOGS FOR PREVENTION OF HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS B

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Background and Aims: Clinical and virological factors associated with occurrence of hepatocellular carcinoma (HCC) in antiviral-treatment-naïve chronic hepatitis B patients have been extensively studied. However, the clinical usefulness of such data is greatly limited because of the wide availability of effective antiviral agents. We aimed to investigate clinical factors associated with HCC development in treatment-experienced patients.
Methods: 331 patients receiving pre-therapeutic liver biopsy were included for outcome assessment. Predictors for HCC occurrence were further analyzed by comparing two age-matched groups receiving different treatment methods.
Results: Cirrhosis and antiviral treatment method [peginterferon-experienced (PE) versus nucleos(t)ide analog-treatment (NAt) only] were significantly associated with subsequent HCC occurrence by initial univariate and multivariate analyses. To further examine this unexpected finding, 120 age-matched patients were retrieved from each of the PE and NAt groups for comparison. Baseline clinical variables showed no statistical difference between the two treatment groups. PE group had a significant longer time to HCC than the NAt group ($P = 0.0091$). The beneficial effect of peginterferon treatment remained when only cirrhotic patients were included ($P = 0.0437$). Additionally, patients receiving peginterferon alone had a significantly longer time to HCC than those receiving entecavir alone ($P = 0.0111$), despite the fact that serum HBV DNA levels of the entecavir-treated subjects were kept lower than those treated by peginterferon alone during the observational period ($P < 0.0001$).
Conclusions: Peginterferon treatment with or without nucleos(t)ide analog is superior to nucleos(t)ide analogs alone for the prevention of HCC occurrence in chronic hepatitis B patients.

P0635
CHRONIC HEPATITIS B TREATMENT INDIVIDUALIZATION BY MEANS OF SERUM HBsAg AND MiR-B-INDEX KINETICS

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Background and Aims: Non-viral biomarkers of sustained control of HBV infection are an unmet need for treatment individualization in Chronic Hepatitis B (CHB). We recently reported that Inactive Carriers (IC) and patients with sustained-virologic-response (SVR) to Peg-IFN share a common serum miRNA signature (MiR-B-Index,

Table (abstract P0635).

	HBsAg (log_{10} IU/mL)						MiR-B-Index					
	BL	T24w	T48w	EOT	PTFU	P**	BL	T24w	T48w	EOT	PTFU	P**
All patients												
SVR (20)	3.62	2.82	2.65	1.95	1.24	<0.001	−5.14	−0.98	1.28	1.63	4.54	<0.001
REL (17)	3.76	3.63	3.53	3.49	3.34	0.013	−9.81	−8.28	−8.50	−7.22	−4.90	0.019
NR (9)	3.96	4.10	4.06	3.66	3.72	0.240	−7.93	−8.73	−10.49	−8.82	−7.04	0.766
*P**	0.451	0.002	<0.001	<0.001	<0.001		0.046	<0.001	<0.001	<0.001	<0.001	
HBeAg positive patients												
SVR (5)	4.97	3.78	−1.52	−1.52	−1.52	0.004	−16.22	3.58	7.23	5.06	2.29	0.011
REL (2)	3.26	3.31	4.08	4.01	2.98	n.a.	−8.88	−6.27	−5.54	−6.52	−3.66	n.a.
NR (4)	4.83	4.78	4.75	4.23	4.39	0.103	−15.33	−14.10	−17.05	−10.68	−11.32	0.382
*P**	0.273	0.144	0.034	0.009	0.010		0.273	0.201	0.034	0.045	0.011	
HBeAg negative patients												
SVR (15)	3.45	2.81	2.69	2.15	1.33	0.002	−4.49	−1.65	1.16	1.46	4.60	<0.001
REL (15)	3.76	3.63	3.50	3.44	3.34	0.008	−9.81	−8.31	−8.80	−7.22	−4.90	0.034
NR (5)	3.70	3.60	3.43	3.58	3.10	0.607	−7.26	−8.29	−7.20	−8.82	−5.92	0.335
*P**	0.042	0.001	<0.001	<0.001	<0.001		0.001	<0.001	<0.001	<0.001	<0.001	

*Mann-Whitney U-test (SVR vs REL+NR); **ANOVA (BL vs EOT).

MBI). We studied the kinetics of MBI and HBsAg during and after Peg-IFN.

Methods: 46 CHB patients (11 HBeAg pos, 29 males, median age 48.6 y, range 22.4–64.1 y; genotypes: A 5, D 39, F 2) were treated with Peg-IFN2a 180 μg/w (median 13.2 months, 8–24 mo): 20 SVR; 17 relapsers (REL) and 9 non-responders (NR). Single RT-q-PCR for miR-122-5p, miR-99a-5p, miR-192-5p, miR-126-3p, miR-335-5p and miR-320a were performed using miRCURY RNA Isolation kit, miRCURY-LNA™ Universal-RT-cDNA-Synthesis and RT-miRNA-PCR (Exiqon-A/S). HBV-DNA and HBsAg were quantified by COBAS TaqMan (Roche) and Architect (Abbott). HBsAg and MBI were tested at baseline (BL), 24–48 wks and end of therapy (EOT) and 24 wks post treatment (PTFU).

Results: Median HBsAg and MBI values at the different time points in overall patients, HBeAg pos and neg are reported in the table. MBI values >−1.7 were found: at BL in 5 HBeAg neg pts (4 SVR, 1 NR); at EOT in 14/19 (73.7%) SVR (4 HBeAg pos, 10 HBeAg neg) and at PTFU in all SVR, but in 1 NR and 1 REL only. In HBeAg neg pts at EOT MBI values ≥−3.0 and HBsAg <1000 IU/mL had 100–93.3% sens., 94.7–85.0% spec., 93.3–82.4% PPV, 100–94.4% NPV and 97.0–88.6% DA in identification of SVR (AUROC 0.988 and 0.951 respectively).

Conclusions: Both HBsAg and MBI show good performances in SVR prediction overall. In HBeAg neg CHB MBI predicts SVR since therapy beginning and identifies all SVR at EOT qualifying as the best biomarker for individual therapy monitoring.

P0636

ANTIVIRAL EFFICACY AND INDUCTION OF HOST IMMUNE RESPONSES WITH SB 9200, AN ORAL PRODRUG OF THE DINUCLEOTIDE SB 9000, IN THE WOODCHUCK MODEL OF CHRONIC HEPATITIS B VIRUS (HBV) INFECTION

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Background and Aims: Activation of the viral sensor proteins, RIG-I and NOD2, by viral nucleic acids results in the production of type I IFNs and subsequent induction of ISGs and antiviral immune cells. SB 9200, an oral prodrug of the dinucleotide SB 9000, activates RIG-I and NOD2 by binding at the nucleotide binding domain of both proteins. This interaction also inhibits the synthesis of viral nucleic acids by steric blockage of the viral polymerase. The host immune stimulating and direct antiviral activities of SB 9200 were evaluated in the woodchuck model of chronic HBV infection. SB 9200 is being developed for the treatment of chronic hepatitis B and C and has completed Phase I clinical trials in treatment-naive HCV-infected patients.

Methods: Two groups of woodchucks with chronic woodchuck hepatitis virus (WHV) infection (n = 5/group) were treated orally, daily with SB 9200 for 12 weeks at doses of 15 or 30 mg/kg. Endpoints included PK, PD, tolerability, and antiviral efficacy.

Results: Treatment with SB 9200 at two select dose levels resulted in up to 4.2 and 2.2 log_{10} reductions in serum WHV DNA or WHV surface antigen (WHsAg) loads, respectively, from pretreatment level. Treatment was also associated with reduced hepatic levels of WHV DNA (up to 41%), WHV cccDNA (up to 32%), and WHV RNA (up to 50%), lower hepatic expression of WHV antigens, and reduced liver disease progression. Following cessation of treatment, recrudescence of WHV replication was observed in all woodchucks treated with 15 mg/kg, whereas woodchucks administered 30 mg/kg had delays in the relapse of serum WHV DNA and WHsAg. The development of an anti-WHs antibody response was not observed. The antiviral effects were associated with a dose-dependent induction of IFNs (IFN-α, IFN-β) and ISGs (OAS-1, CXCL10, ISG15) in blood and liver. Prolonged SB 9200 administration at both dose levels was well tolerated with CBC, hematology and serum biochemistry parameters all appearing normal through the treatment period.

Conclusions: Oral administration of SB 9200 for 12 weeks in woodchucks with chronic WHV infection resulted in marked reductions in serum and hepatic levels of viral DNA, RNA, and antigens that were associated with (or were a result of) the induction of host immune responses. These results suggest that SB 9200 can induce an antiviral immune response during chronic active hepadnaviral infection that has the potential for a functional cure in the treatment of chronic hepatitis B, most likely in combination with approved anti-HBV drugs.

P0637

SHORT-TERM ANTIVIRAL PROPHYLAXIS IS NOT EFFECTIVE IN HBsAg-NEGATIVE, ANTI-HBc POSITIVE, AND/OR ANTI-HBs PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background and Aims: Hepatitis B virus (HBV) reactivation can occur in persons who are hepatitis B surface antigen (HBsAg)-negative but hepatitis B core antibody (anti-HBc) positive, especially in patients receiving bone marrow or stem cell transplantation.

However, data are scarce regarding universal use of the prophylactic antiviral agents or optimal duration of prophylaxis to date. In this study, we compared the frequency of hepatitis B reactivation between prophylactic group and non-prophylactic group in hematopoietic stem cell transplantation patients.

Methods: From January 2008 to December 2013, a total of 315 HBsAg-negative, anti-HBc positive patients were enrolled who receive autologous or allogenic stem cell transplantation. Patients were divided into prophylactic and non-prophylactic groups. The primary endpoint was the incidence of HBV reactivation, which was defined as a increase in HBV DNA 10 times than baseline or HBsAg-reverse seroconversion.

Results: The median age of patients was 49.7 years (range, 16–71), and 181 (57.5%) were male. Among the patients, 219 (69.5%) did not take prophylaxis, and 96 (30.5%) underwent prophylaxis as follows: 90 to telbivudine; 5 to entecavir; 1 to lamivudine, respectively. Of these, 12 patients developed HBV reactivation (prophylactic group, 4; non-prophylactic group, 8). The median time to reactivation was 20.5 months (range, 10.0–39.0) after the initiation of chemotherapy. All of the patients were successfully treated with telbivudine, entecavir or tenofovir, and none of them experienced liver-related death. The risk of reactivation was not different between prophylactic and non-prophylactic group ($P = 0.061$).

Conclusions: HBV reactivation in HBsAg-negative, anti-HBc positive patients undergoing hematopoietic stem cell transplantation is not rare. However, all cases were successfully treated with on-demand antiviral agents without serious liver problems, including hepatic failure and death. Antiviral prophylaxis against HBV reactivation was not effective, especially in the short-term duration (<12 months). Therefore, careful monitoring of HBV DNA and on-demand antiviral treatment would be safe and cost-effective rather than routine prophylaxis in these patients.

P0638

GLOBAL EPIDEMIOLOGY OF HEPATITIS DELTA: FIRST DATA FROM THE HEPATITIS DELTA INTERNATIONAL NETWORK

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Background and Aims: Hepatitis D virus (HDV) infection is the most severe form of chronic viral hepatitis associated with accelerated fibrosis progression and an increased risk for developing liver-related clinical complications. However, the clinical and virological presentation of hepatitis delta patients varies largely between different regions and countries. The Hepatitis Delta International Network (HDIN) was established in 2011 and granted with an EASL registry award in 2014.

Methods: The primary aim of the HDIN registry is to establish a large global data base of patients chronically infected with HDV to better define the course of hepatitis delta and response to antiviral therapy in the context of different HDV and HBV genotypes and diverse host genetic and environmental backgrounds. A structured eCRF optimized for hepatitis delta was implemented and 12 centers world-wide are participating. We here report data of the first 674 patients included until November 1st 2014.

Results: 85% of patients were HDV RNA positive, 75% HBeAg-negative and 37% had liver cirrhosis with 10.9% reporting an episode of previous hepatic decompensation. Patients were divided according to the country of birth into Eastern Mediterranean (EM, n = 152), Eastern Europe and Central Asia (EE/CA, n = 158), Central and Southern Europe (CE, n = 40), South Asian (SA, mainly Pakistan; n = 242) and Africa/South America (AF/SA, n = 50). The mean patient age differed largely between regions with patients from SA being more than 10 years younger than EE/CA and EM patients (SA mean age 35.9 years vs. EE/CA 47.0 years and EM 49.7 years) while CE patients were oldest with a median age of 54.1 years ($P < 0.001$). Gender distribution of patients included in the registry differed largely with patients being male ranging from 46.8% (EE) to 82.4% (CA). Previous antiviral therapy was reported for only 37% of patients (48–70% for European patients vs. 33.5% SA vs. Brazil 5%). Patients receiving polymerase inhibitors vary from 2.48% (SA) to 13.82% (EM) and different types of IFNa from 4% (AF/SA) to 22.15% (EE/CA). Liver cirrhosis was most frequent in CE and EM patients (47.5% and 52.63% vs. 28.93% SA, 31% EE/CA and 12% AF/SA).

Conclusions: The HDIN registry confirms the severity of chronic hepatitis delta but also highlights the diversity of patient characteristics in different regions world-wide – possibly requiring different management strategies. More detailed data on virological and clinical characteristics will be presented at the ILC.

P0639

TELBIVUDINE VERSUS TENOFOVIR-BASED TREATMENT OPTIMISATION STRATEGY IN HBeAg-NEGATIVE CHRONIC HEPATITIS B PATIENTS

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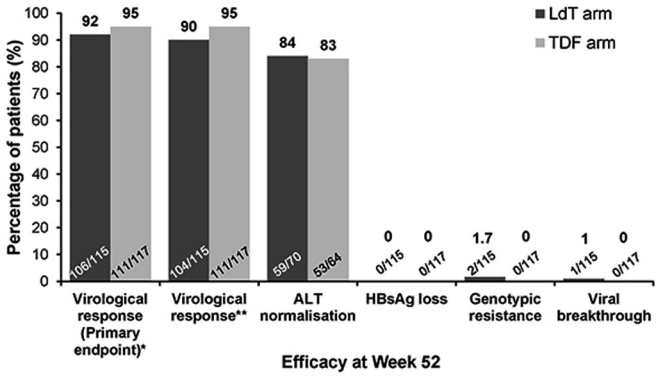
Background and Aims: Chronic hepatitis B (CHB) is a common global health problem. Early virological response with antiviral treatment with telbivudine (LdT), particularly at week 24, is associated with better long-term treatment outcomes in CHB patients. This study evaluated the treatment outcomes of conditional intensification of LdT monotherapy with tenofovir (TDF) in HBeAg-negative CHB patients.

Methods: Patients were randomised (1:1) to receive LdT 600 mg q.d. or TDF 300 mg q.d. in this prospective, open-label study. After LdT or TDF monotherapy for 24 weeks, patients with HBV DNA ≥300 copies/mL received TDF/LdT add-on therapy until week 104, whereas those with <300 copies/mL continued monotherapy. The modified intent-to-treat (mITT) population comprised patients who did not discontinue before and had not received add-on therapy at week 24.

Results: Of the 241 randomised patients (LdT, 121; TDF, 120), 232 (LdT, 115; TDF, 117) were included in the mITT population. Overall, 80% and 89.7% of patients on LdT and TDF had early virological response (HBV DNA level <300 copies/mL) at week 24. In total, 92% and 95% of patients on LdT and TDF achieved HBV DNA

levels <300 copies/mL at week 52; primary efficacy endpoint for non-inferiority (margin −10%) was met [difference in percentages (95% CI): −2.9% (−9.1, 3.4)] (figure). Among those patients who were suboptimal responders (HBV DNA ≥300 copies/mL) at week 24 and received add-on treatments, HBV DNA level decreased to <169 copies/mL at week 52 in 82% (LdT+TDF) and 91% (TDF+LdT) of patients. The percent change in estimated glomerular filtration rate (eGFR) from baseline was significantly greater in LdT monotherapy compared with TDF monotherapy at week 52 (4.21 versus −3.44; p = 0.0053) and at week 104 (6.14 versus −3.00; p < 0.0001). Compared with TDF monotherapy, twice as many patients on LdT monotherapy (60.5% LdT versus 27.5% TDF) with abnormal eGFR at baseline reverted to normal at week 104. Serious adverse events deemed unrelated to study treatment were reported in 17 patients (LdT, 8; TDF, 9). One patient in the LdT arm discontinued due to myalgia and four patients in the TDF arm discontinued due to cholestatic jaundice, hepatocellular carcinoma, hepatic cirrhosis and headache.

Conclusions: Data from this head-to-head randomised clinical trial showed that LdT was well tolerated and non-inferior to TDF for treating HBeAg-negative CHB. LdT was associated with improvement in eGFR compared with TDF.



*HBV DNA <300 copies/mL at Week 52. Difference in percentages (95% CI): -2.9% (-9.1, 3.4).
**HBV DNA <169 copies/mL at Week 52. Efficacy analysis was done for the modified intent-to-treat population.
ALT: alanine aminotransferase; HBsAg: hepatitis B surface antigen; LdT: telbivudine; TDF: tenofovir disoproxil fumarate

Figure: Efficacy outcomes of LdT versus TDF.

P0640

EFFECT OF THE COMBINATION OF THE HBV CORE INHIBITOR NVR 3-778 WITH NUCLEOSIDE ANALOGS OR OTHER HBV CORE INHIBITORS ON THE INHIBITION OF HBV DNA REPLICATION IN HepG2.2.15 CELLS

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Background and Aims: NVR 3-778 represents a new class of HBV core inhibitors and is in clinical development for the treatment of chronic hepatitis B. The availability of NVR 3-778 as an HBV replication inhibitor with a new mechanism of action allows the assessment of the potential for increased antiviral potency that could be achieved in combination treatment with other antiviral agents. Different classes of HBV core inhibitors may also show benefit in combination.

Methods: HepG2.2.15 cells contain stably integrated HBV DNA and generate infectious HBV particles. These cells were used to investigate the interaction between NVR 3-778 and nucleoside analogs Lamivudine (LMV), Tenofovir (TFV) and Entecavir (ETV). The combination of NVR 3-778 with another core inhibitor, NVR 3–7484 was also assessed. The reduction of HBV DNA in the cell culture supernatant was determined after cells were incubated with different concentrations of NVR 3-778 and a nucleoside analog or other core inhibitor in a checkerboard format for 6 days. Cell viability was assessed by the determination of intracellular ATP concentrations. MacSynergy II was used to evaluate the effect of compound combinations.

Results: HBV DNA replication was monitored by measuring levels of secreted, encapsidated HBV DNA in HepG2.2.15 cell supernatant. NVR 3-778, NVR 3–7484, LMV, TFV and ETV inhibited HBV DNA replication with mean EC_{50} values of 0.47 μM, 0.52 μM, 0.11 μM, 1.4 μM and 3.2 nM, respectively. Combining NVR 3-778 with each of the three nucleoside analogs resulted in additive antiviral effects. We also observed an additive antiviral effect from the combination of two HBV core inhibitors, NVR 3-778 and NVR 3–7484. Cell viability remained above 90% in all samples treated with the highest compound concentrations, either alone or in combination.

Conclusions: The combination of the HBV core inhibitor NVR 3-778 with nucleoside analogs lamivudine, tenofovir or entecavir showed additive antiviral effects against HBV replication without reductions in cell viabilty. In addition, the combination of two different HBV core inhibitors showed additive antiviral activity. These results support further exploration of the benefit of such combinations in intensifying the overall suppression of HBV replication and production of HBV from infected cells.

P0641

EXCELLENT 5-YEAR SURVIVAL IN CAUCASIAN CHRONIC HEPATITIS B (CHB) PATIENTS WITH OR WITHOUT CIRRHOSIS UNDER LONG-TERM ENTECAVIR (ETV) OR TENOFOVIR (TDF) THERAPY AND THE IMPACT OF HEPATOCELLULAR CARCINOMA (HCC)

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Background and Aims: Long-term ETV/TDF therapy represents the most common treatment option in CHB of any severity, but efficacy data have been mainly based on on-therapy virological remission rates. In this 9-center, large ongoing cohort study, we evaluated the survival in Caucasian CHB patients with or without cirrhosis who have been receiving ETV/TDF.

Methods: We included 1815 adult Caucasians with CHB with or without compensated cirrhosis and no HCC at baseline (mean age: 53±14 years, males: 71%, naive to antivirals: 60%, cirrhosis: 28%) who received ETV/TDF for ≥12 months. Liver transplantation free survival rates were estimated from Kaplan–Meier curves.

Results: In the total patient population, 1-, 2-, 3- and 5-year overall survival rates were 99.5%, 98.5%, 97.5% and 95% being significantly higher in non-cirrhotics (100%, 99%, 98.5% and 97%) than cirrhotics

(98%, 96.5%, 95% and 92%, P < 0.001). When only liver related deaths or LT were taken into account, 1-, 2-, 3- and 5-year survival rates were 99.7%, 99.4%, 99% and 97.5% being also significantly higher in non-cirrhotics (100%, 100%, 100% and 99%) than cirrhotics (99%, 98%, 97% and 95%, P < 0.001). After excluding patients who developed HCC, 1-, 2-, 3- and 5-year overall survival rates were 99.5%, 99%, 98% and 96.5% remaining significantly higher in non-cirrhotics (100%, 99%, 99% and 98%) than cirrhotics (98%, 97.5%, 96.5% and 94%, P = 0.001). In the 85 patients with HCC, the 1- and 3-year overall survival rates after HCC diagnosis were 76% and 56% without any difference between non-cirrhotics and cirrhotics (P = 0.997). In multivariable Cox regression analysis, better overall survival was independently associated with absence of HCC [HR: 6.446 (95% CI: 3.647–11.394), P < 0.001] and younger age [HR per year: 1.043 (1.019–1.069), P = 0.001] but not with cirrhosis (P = 0.085) or gender (P = 0.438), while better liver related survival was associated only with absence of HCC [HR: 22.451 (9.891–50.780), P < 0.001] and not with age (P = 0.823), cirrhosis (P = 0.123) or gender (P = 0.970).

Conclusions: The survival of Caucasian CHB patients treated with ETV/TDF is excellent with >95% of cases surviving at 5 years and a significant proportion of deaths coming from liver unrelated causes. HCC development is a major factor affecting the overall mortality and the only factor affecting liver related mortality in such patients.

P0642

THE EFFICACY AND SAFETY OF TELBIVUDINE OR TENOFOVIR DISOPROXIL IN PREGNANCY FOR PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HEPATITIS B VIRUS

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Background and Aims: This study was to evaluate the efficacy and safety of telbivudine (LdT) and tenofovir (TDF) as monotherapy or combined treatment in HBeAg-positive pregnant women with high viral load for preventing mother-to-child transmission (MTCT) in an open-labeled study.

Methods: The 355 HBeAg-positive pregnant women with HBV DNA >10^6 IU/mL enrolled were treated with antiviral therapy from 24–32 weeks of gestation (308 in LdT group, 23 in TDF group, 24 in TDF+LDT group). All infants were vaccinated with recombinant HBV vaccine and hepatitis B immune globulin (HBIG) according to standard immunoprophylaxis procedure. MTCT rate was determined by HBsAg and HBV DNA detection in 6 months after birth.

Results: Significantly lower HBV DNA levels were noted in all the enrolled mothers when delivery. The rate of HBsAg positive and HBV DNA detectable in infants at 6 months was 0% in each group. The incidence of undetectable cord blood HBV DNA levels has no significantly difference among the three groups (LdT group, 99.2%; TDF group, 100%; LdT+TDF group, 100%; P > 0.05). No severe adverse events, including blood creatine kinase (CK) and estimated glomerular filtration rate (eGFR), or complications were observed in all the mothers and infants.

Conclusions: LdT and TDF monotherapy or their combo-therapy were equally effective and well-tolerated in HBeAg positive and high viral load mothers as well as their infants on short term follow up, and they were associated with significant reduction of MTCT.

P0643

SERUM HBV RNA IS AN EARLY PREDICTOR OF HBeAg SEROCONVERSION IN PATIENTS WITH CHRONIC HEPATITIS B (CHB) TREATED WITH PEG-INTERFERON ALPHA-2A (40kD)

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Background and Aims: HBeAg seroconversion (SC) is a desired treatment outcome of current therapies in HBeAg positive CHB patients. However, this outcome is achieved only in a minority of treated patients and current biomarkers (e.g. HBsAg, HBV DNA) are limited in being able to identify patients that will ultimately respond to treatment. We explored weather serum HBV RNA levels can be used to predict HBeAg SC before or during PegIFN alfa-2a treatment.

Methods: Eighty-nine patients with HBeAg positive CHB, from two large phase III / IV PegIFN alfa-2a trials, were retrospectively studied. Patients were treated with Peg-IFN alpha-2a 180 μg/week alone (n = 58) or in combination with lamivudine 100 mg/day (n = 32) for 48 weeks. Clinical data was obtained from the completed trials and provided by Roche. After reverse transcription, polyadenylated HBV RNA was quantified based on a specific real-time PCR from serum samples collected at baseline, at weeks 12 and 24 of treatment. Response was defined as HBeAg SC occurring 24 weeks after stopping treatment. Differences in HBV RNA concentrations were assessed by Student's t-tests.

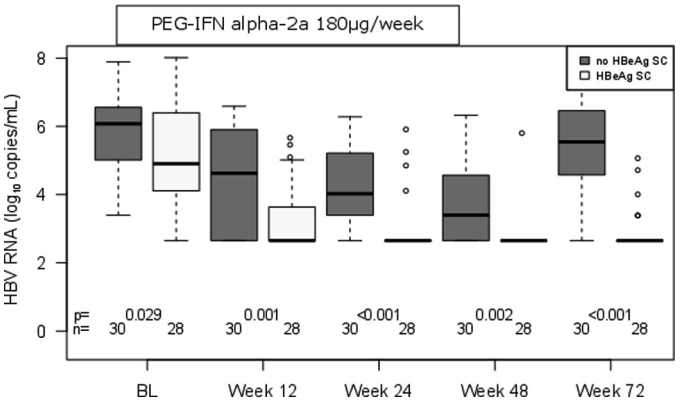

Figure 1. HBV RNA in patients treated with PegIFN alfa-2a monotherapy.

Results: In 58 patients treated with PegIFN alfa-2a alone (Figure 1), baseline HBV RNA were significantly lower in responders (n = 28) compared to non-responders (n = 30) (mean 5.2±1.4 [2.7–8.0] vs. 5.9±1.1 [3.4–7.9] $\log_{10}$ copies/mL, p = 0.029). Responders continued to show significantly lower HBV RNA levels during treatment, at weeks 12 and 24 (mean 3.2±0.9 [2.6–5.7] vs. 4.2±1.4 [2.6–6.5] $\log_{10}$ copies/mL, p = 0.001 and 2.9±0.7 [2.7–5.9] vs. 4.0±1.3 [2.7–6.3] $\log_{10}$ copies/mL, p < 0.001, respectively).

Conversely, in 32 patients treated with PegIFN alfa-2a + lamivudine, HBV RNA levels at baseline and week 12 were not significantly different between responders (n = 13) and non-responders (n = 19) (mean 5.1±1.0 [2.7–6.3] vs. 5.7±1.0 [3.4–7.4] $\log_{10}$ copies/mL, p = 0.185 and 3.1±0.5 [2.7–4.0] vs. 3.5±1.0 [2.7–5.6] $\log_{10}$ copies/mL, p = 0.185, respectively). However, at week 24 HBV RNA had become undetectable in all responders whilst it remained present in the majority of non-respodners (3.1±0.8 [2.7–4.9] $\log_{10}$ copies/mL, p = 0.018).

Conclusions: Serum HBV RNA levels is a novel baseline and on-treatment biomarker for early prediction of serological response to PegIFN alpha-2a treatment in patients with HBeAg positive CHB.

P0644

HCC RISK SCORES: APPLICATION OF THE CU-HCC, GAG-HCC AND PAGE-B SCORES TO CHRONIC HEPATITIS B (CHB) PATIENTS TREATED WITH TENOFOVIR DISOPROXIL FUMARATE (TDF)

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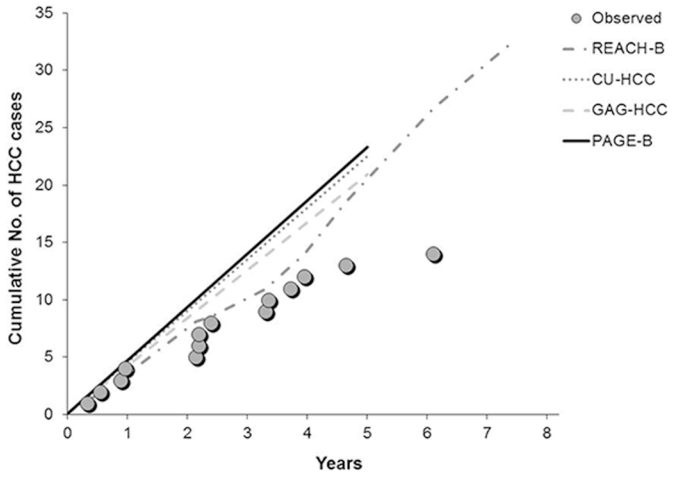
Background and Aims: Hepatocellular carcinoma (HCC) remains a dreaded complication of CHB. Several nomograms have been developed to assess a CHB patient's risk of developing HCC. We have previously applied the REACH-B score to our population. Here we used additional HCC nomograms to assess the impact of therapy on HCC incidence.

Methods: We applied the CU-HCC, GAG-HCC (modified score not requiring viral mutation data), and PAGE-B risk scores to patients enrolled in pivotal trials GS-US-174-0102 (HBeAg−) and GS-US-174-0103 (HBeAg+) for tenofovir disoproxil fumarate (TDF) to assess the impact of therapy on HCC incidence. All 3 scores provide projections at 5 years, with the first two scores also providing projections at 10 years. Given that 384 weeks (7.4 years) of actual observed data are available for Studies -0102/0103, we compared the number of observed HCC cases in the study with what the prediction models estimated for the first 5 years of follow-up.



Results: Among the 641 patients who entered pivotal studies GS-US-174-0102 (HBeAg-) and GS-US-174-0103 (HBeAg+), 14 cases of HCC occurred during the first 384 weeks (7.4 years, n = 404) of the studies. At the end of year 5 (n = 472), CU-HCC predicted 22.5 cases, GAG-HCC predicted 20.9 cases and PAGE-B predicted 23.3 cases. Predicted cases HCC along with all actual observed cases through 7.4 years are provided in the figure. For comparison, our previous calculations with REACH-B predicted 20.8 cases after 5 years and 32.2 by the end of follow up and are also provided in the figure.

Conclusions: In a mixed-genotype population comprised of 75% non-Asians, all 3 risk scores predicted similar numbers of HCC cases at the end of 5 years, which was consistently higher than that observed among TDF study participants. Despite the potent viral suppression by TDF, the risk of HCC is not eliminated, necessitating continued monitoring for HCC.

P0645

NON-SYNONYMOUS SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPS) IN TLR7 DO NOT IMPACT GS-9620 DEPENDENT TLR7 ACTIVATION

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Background and Aims: Activation of TLR7 constitutes an important host defense mechanism against viral pathogens. GS-9620, an oral small molecule agonist of toll-like receptor 7 (TLR7), is being evaluated in phase 2 clinical trials for the treatment of chronic hepatitis B (CHB). The overall objective of the study was to gain insight into the molecular basis for GS-9620 activation of TLR7 and assess the functional impact of a number of non-synonymous single-nucleotide polymorphisms (SNPs) in TLR7. A combination of structure-based modeling, mutational analysis and cell biology was used to determine the cellular localization of GS-9620 binding to TLR7, the key binding residues of GS-9620 in TLR7, and the effect of common TLR7 SNPs on GS-9620-mediated responses.

Methods: Intracellular accumulation and sub-cellular distribution studies of radiolabelled GS-9620 were performed in the Daudi B cell line. Plasmacytoid dendritic cells (pDCs) were isolated from peripheral blood mononuclear cells and treated with Bafilomycin A to disrupt endolysosomal pH. Interferon-alpha (IFN-α) was measured by ELISA. Mapping of the GS-9620 binding site was performed by transient transfections of TLR7 harboring amino acid point mutations and measurement of NF-κB dependent luciferase reporter activity in Huh7, a human hepatoma cell line. Co-immunoprecipitation and western blotting techniques were used for receptor dimerization studies.

Results: GS-9620 rapidly and preferentially localized to, and accumulated in, lysosomes. Bafilomycin A treatment inhibited the induction of IFN-α in pDCs by GS-9620, suggesting that GS-9620 required endolysosomal acidification to activate TLR7. We also showed that TLR7 exists as preformed dimers prior to ligand binding. Consistent with structure-based modeling predictions, our data indicate that the TLR7 dimer interface provides a binding pocket for GS-9620. In particular, residues Y356, F408, D555, L557 and T586, which reside within the predicted binding pocket of TLR7, proved critical for GS-9620 activation of TLR7. Importantly, we found that the reported non-synonymous TLR7 SNPs rs179008-Q11, rs55907843-V222 and rs5743781-A448 did not affect GS-9620-dependent activation of TLR7.

Conclusions: These data indicate that GS-9620 binds to TLR7 dimers at their interface in the endo-lysosomal compartment to trigger TLR7-dependent responses. We demonstrate that prevalent SNPs in TLR7 have no detectable impact on GS-9620-dependent TLR7 activation.

P0646

LOWER RISK OF HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS B PATIENTS TREATED WITH ENTECAVIR: A REACH-B ANALYSIS OF THE ENUMERATE STUDY

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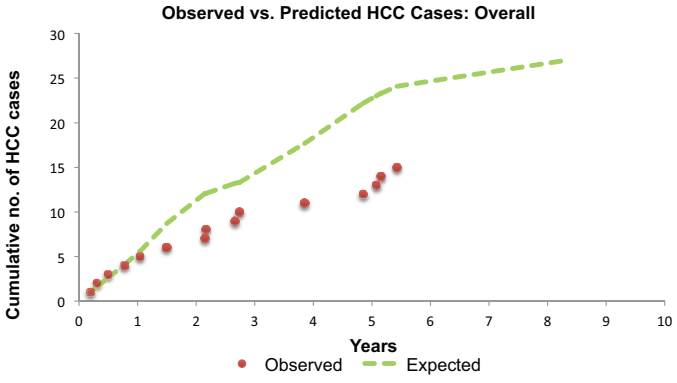
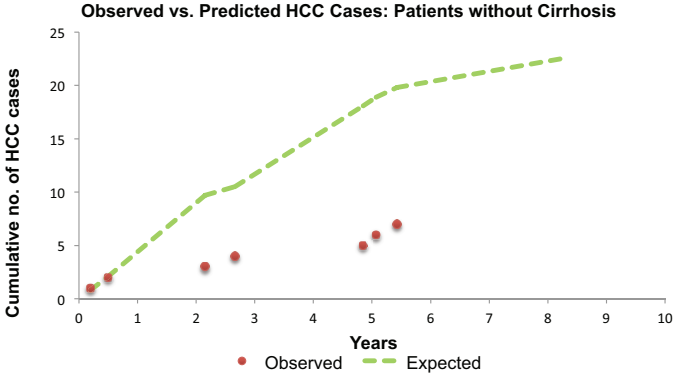
Background and Aims: Entecavir (ETV) has been shown to be associated with decreased hepatocellular carcinoma (HCC) incidence in patients with chronic hepatitis B (CHB). However, data from large U.S. cohorts, especially in patients without cirrhosis, is limited.
Aims: To compare the observed HCC incidence in a large multicenter U.S. cohort of CHB patients treated with ETV with the predicted incidence as estimated by the REACH-B HCC scoring system.
Methods: The ENUMERATE study was conducted in a national network of 26 academic and community liver centers in the U.S., in partnership with the AHF. The cohort included treatment-naïve CHB patients ≥18 years old who did not have a prior history of HCC and were treated with ETV for ≥12 months between 2005 and 2013. Exclusion criteria included co-infection with HIV, hepatitis C or D virus, or a history of solid organ transplantation. The primary outcomes were observed new HCC diagnosis, confirmed by AASLD criteria, and standardized incidence ratios (SIR) between the observed and predicted numbers of HCC cases. The predicted HCC incidence was calculated using the REACH-B model (Yang et al. Lancet Oncology 2011).





Results: The ENUMERATE cohort included 745 patients. Of these, 577 patients had known cirrhosis status, baseline HBV DNA, ALT, and HBeAg status and were included in the REACH-B analysis. Mean age was 45.7, 64% were men, 83% were Asians, 41% were HBeAg+, and 9.4% had cirrhosis. The overall cumulative 5-year HCC incidence was 2.0% in patients without cirrhosis and 23.2% in those with cirrhosis. During a median follow-up of 4 (1–8.3) years, 15 patients developed HCC: 8 out of 54 patients with cirrhosis and 7 out of 523 patients without cirrhosis. The estimated number of HCC cases for this cohort as predicted by the REACH-B calculation was 26.9 overall and 22.5 among those without cirrhosis. The SIR was 0.56 (95% confidence interval [CI] 0.34–0.92) overall and 0.31 (95% CI 0.15–0.65) for those without cirrhosis.
Conclusions: The observed incidence of HCC in patients treated with ETV was lower than that predicted using the REACH-B model for both the overall cohort and for patients without cirrhosis. These findings suggest that ETV can lower HCC risk even among patients without cirrhosis. However, antiviral therapy does not eliminate the risk of HCC in patients with CHB and careful surveillance for HCC remains warranted in these patients notwithstanding ETV treatment.

P0647

PROACTIVE DOSE ADJUSTMENTS ARE NECESSARY IN MANY ADV-EXPERIENCED PATIENTS TREATED WITH TDF MONOTHERAPY FOR 5 YEARS: A PROSPECTIVE COHORT STUDY IN 320 PATIENTS

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Background and Aims: Tenofovir (TDF) is a popular anti-HBV strategy for NUC-experienced patients, but its safety profile in field practice patients previously exposed long-term to Adefovir (ADV) is under discussion because of recent reports of Fanconi syndrome
Methods: 320 NUC-experienced CHB patients received Tenofovir (TDF) for 69 months (range 1–106) as a switch from ADV+LAM or as a rescue therapy for LAM, ADV or ETV. Baseline features were: age 59 (24–82), 85% HBeAg-negative, 62% cirrhotics, 88% normal ALT, 74% undetectable HBV-DNA, 86% switched from ADV+LAM. Virological response was undetectable HBV DNA; safety analysis focused on glomerular (eGFR) and tubular renal function. Baseline was defined as the start of TDF.
Results: During 5 years of TDF, 89 patients (28%) had to reduce TDF dose after 31 months (2–75); 11 additional patients (3.4%) had to stop TDF because of renal side effects, and 6 (2%) withdrew for gastrointestinal side effects. TDF dose was reduced in 57 patients due to eGFR decline, in 30 patients due to phosphate decline and in 2 cases for both events. All patients who had to stop TDF had low blood phosphate levels. Overall, 106 patients (33%) either required a dose reduction or withdrew from TDF for side effects, with a 5 year estimated probability of 40%. Most of those patients who had to stop TDF were successfully rescued by switching to ETV. Of note, none of the TDF treated patients developed acute renal failure, or Fanconi syndrome and viral load did not rebound in any patient who required dose adjustment. Virological response progressively increased to 100% at year 5 with most patients achieving normal ALT. 15 patients (5%) cleared HBsAg, 26 additional patients had qHBsAg <10 and 25 reached qHBsAg between 10 and 100 IU/ml (Overall, 22% of the patients had qHBsAg <100 IU/ml). HCC attack rates were 1.3%/year in compensated cirrhotics and 0.2%/year in non cirrhotics, which were significantly lower than those described in untreated and in TDF treated naïve patients. No cases of clinical decompensation without HCC were recorded.

Overall, 7 patients (2%) were transplanted (all for HCC) and 16 (5%) died (7 for HCC, 7 for non liver causes and 2 for unknown reasons).
Conclusions: To prevent glomerular and tubular damage, proactive dose adjustments of TDF are necessary in a large proportion of ADV-experienced, TDF treated CHB patients. Current EASL guidelines on safety management and monitoring of these patients must be revised.

P0648
LONG-TERM OUTCOME OF CHEMOTHERAPY-INDUCED HBV REACTIVATION IN LYMPHOMA PATIENTS WITH RESOLVED HBV INFECTION

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Background and Aims: Hepatitis B virus (HBV) reactivation can occur to 10–40% of lymphoma patients with 'resolved' HBV infection (HBsAg-negative and anti-HBc-positive) who received rituximab-based chemotherapy, but its impact on survival outcome was unclear.
Methods: We prospectively followed 150 newly diagnosed lymphoma patients with resolved HBV infection who receive rituximab-CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone)-based chemotherapy by regular HBV DNA monitoring. Patients with documented HBV reactivation, defined as a greater than 10-fold increase in HBV DNA compared with previous nadir levels, were treated with entecavir. Quantification of anti-HBc and anti-HBs levels at baseline was correlated with the risk of HBV reactivation and survival.
Results: With a median follow-up of 46.0 months (range 1.1–63.9), patients who had HBV reactivation after rituximab-CHOP chemotherapy (n = 17) had worse survival than patients without (n = 133). The estimated 3-year overall survival rate was 52.3% (95% CI 50.2–54.4%) for patients with HBV reactivation and 78.2% (95% CI 72.3–84.1%) for those without (p = 0.033), and the 3-year progression-free survival rate was 46.3% (95% CI 39.9–52.7%) vs. 72.9% (95% CI 69.6–76.3%) (p = 0.062). Thirty-eight patients died during follow-up. The major causes of death were tumor progression (18 patients), infection/sepsis (17 patients) and others (3 patients) and not significantly different between patients with or without HBV reactivation. Low anti-HBs and high anti-HBc levels at baseline predicted high risk of HBV reactivation.
Conclusions: Chemotherapy-induced HBV reactivation was associated with worse survival in lymphoma patients with resolved HBV infection. Patients with low baseline anti-HBs and high anti-HBc levels may benefit most from prophylactic antiviral therapy.
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P0649
CHARACTERIZATION OF HBsAg LOSS IN PATIENTS WITH CHRONIC HEPATITIS B (CHB) TREATED WITH NUCLEOS/TIDE ANALOGS (NUCS): A RETROSPECTIVE MULTICENTER STUDY (HEBESAS)

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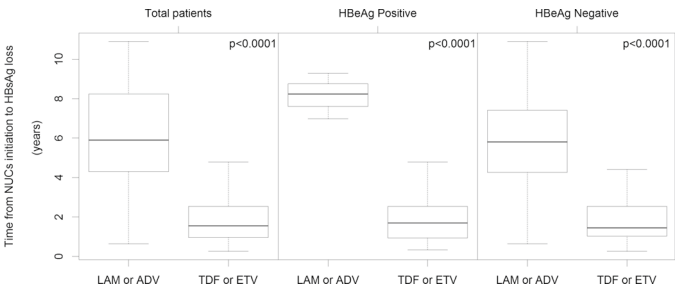
Background and Aims: HBsAg loss is the optimal goal of CHB treatment but it is difficult to achieve in patients treated with NUCs, mainly in HBeAg negative patients. The aim of this study was to investigate the patient characteristics associated with HBsAg loss.
Methods: Retrospective multicenter study of CHB patients treated with NUCs that lost HBsAg after 2007. Exclusion criteria were spontaneous or occurred during IFN/PEG-IFN therapy HBsAg loss, HCV, HDV or HIV coinfection, liver transplantation or HBV reactivation due to immunosuppressive therapies.
Results: A total of 86 patients were included, 91% male and 94% Caucasian, median age of 51 years at the time of HBsAg loss. 53% (45/85) were HBeAg positive at the start of NUCs. Genotype was available in 32 patients, the majority being D (44%) and A (34%). Severe fibrosis or cirrhosis was present in 31% (15/49).
HBV DNA was persistently undetectable in 69% patients before HBsAg loss: median duration 1.19 yr in HBeAg positive and 2.97 yr in HBeAg negative patients (p = 0.014). Upon HBsAg loss, HBV DNA was detectable (52 IU/ml) only in one patient and 34% (25/73) had developed antiHBs. The time between diagnosis of CHB and HBsAg loss was significantly longer in HBeAg negative than in HBeAg positive patients: median, 10.5 vs. 3.1 yr, respectively (p < 0.0001). Although the time from NUCs initiation to HBsAg loss differed between HBeAg negative and HBeAg positive patients (median, 3.2 vs. 1.9 yr, respectively), it did not reach statistical significance (p = 0.086).
HBsAg loss occurred in 62.8% patients treated with TDF (39.5%) or ETV (23.3%), followed by LAM (15.1%) and ADV (9.3%). The mean time from NUCs initiation to HBsAg loss was 1.8 yr with ETV/TDF and 6.2 yr with ADV/LAM (difference was 4.4 yr, CI: 3.5–5.3, p < 0.0001) [Box plot].



Treatment was discontinued in 75.6% patients after HBsAg loss (median, 8.7 months). HBV DNA was undetectable in all patients

and 59% (36/61) had developed antiHBs at the time of NUCs discontinuation. No HBsAg seroreversion occurred during a median of 12 months follow-up (range, 2–55 months). No patient developed hepatic complications or hepatocellular carcinoma.
Conclusions: These data suggest the time between the diagnosis of CHB and HBsAg loss is longer in HBeAg negative than in HBeAg positive patients. In addition, HBsAg loss occurred faster with TDF/ETV than with LAM/ADV. HBsAg loss was maintained in all patients after discontinuation of NUCs.

P0650
RENAL AND BONE EVENTS IN PATIENTS WITH CHRONIC HEPATITIS B RECEIVING LONG-TERM ORAL NUCLEOS(T)IDE ANALOGUE TREATMENT – A STUDY OF 53,500 SUBJECTS

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Background and Aims: Oral nucleos(t)ide analogues (NAs) effectively suppress hepatitis B virus (HBV) and reduce hepatic events in patients with chronic hepatitis B (CHB). Early data suggest nucleotide analogues (adefovir and tenofovir) may cause renal and bone toxicity, but the observation was only based on small number of patients and surrogate endpoints. Long-term data on hard clinical outcomes are lacking.
Methods: We conducted a territory-wide cohort study using the database from Hospital Authority, which provides medical services at both in-patient and out-patient settings for 70–80% of the Hong Kong citizens. We identified CHB patients by International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnosis codes, diagnosed between 2000 and 2012. Other relevant diagnoses, with special focus on renal failure and fracture, procedures, concomitant drugs, and laboratory parameters were retrieved and studied. The primary outcome was incident chronic renal failure and fracture. A 3-year Landmark analysis, with follow-up duration up to 7 years, was used to evaluate the relative risk of primary outcome in patients with or without NA treatment. Baseline covariates of the two groups were balanced using inverse probability of treatment weighting (IPTW) based on propensity score methods.
Results: 53,500 CHB patients (46,454 untreated and 7,046 treated) were included in the analysis. At a median follow-up of 4.9 years, chronic renal failure, renal replacement therapy, all fractures, hip fractures and spine fractures occurred in 270 (0.6%), 96 (0.2%), 318 (0.7%), 48 (0.1%) and 53 (0.1%) of untreated subjects; and 100 (1.4%), 49 (0.7%), 95 (1.3%), 17 (0.2%) and 15 (0.2%) of treated subjects. Male gender and age ≥50 years were associated with these events. After propensity score weighting, NA therapy did not increase the risk of the events (hazard ratios [HR] ranged from 0.79 to 1.31; P values ranged from 0.225 to 0.887). Exposure to nucleotide analogues, compared with nucleoside analogues, did not increase the risk of all events (HR ranged from 0.47 to 1.37; P values ranged from 0.148 to 0.893), except hip fracture (HR = 4.71, 95% confidence interval 1.67–13.3, P = 0.003). Similar findings were observed when comparing nucleotide analogues with no treatment.
Conclusions: NA treatment does not increase the risk of renal and bone events in general. Nucleotide analogues may increase the risk of hip fracture, but the overall event rate is low.

P0651
CLINICAL OUTCOME AND PREDICTOR FOR RELAPSE AFTER CESSATION OF ORAL ANTIVIRAL TREATMENT IN CHRONIC HEPATITIS B PATIENTS: A PROSPECTIVE OBSERVATIONAL COHORT STUDY

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Background and Aims: Little is known about stopping rules of nucleos(t)ide analog (NA) treatment for chronic hepatitis B (CHB).
Methods: A total of 164 consecutive CHB patients who met cessation criteria of NA treatment by APASL guideline were enrolled in this prospective study. Fifty-one patients were excluded by exclusion criteria, 113 patients (45 HBeAg-positive and 68 HBeAg-negative CHB), who stopped NA treatment, remained for statistical analysis. At the time of discontinuing NA, the blood parameters including biochemistry, HBV-DNA, HBsAg, IP-10, and HBcrAg level was obtained and thereafter the patients were monitored regularly with HBV-DNA and ALT. Relapse was defined as reappearance of HBV-DNA >2000 IU/mL after stopping NA treatment.
Results: Within 1 year after NA treatment, relapse occurred in 26 (57.8%) of 45 HBeAg-positive patients with median time of 219 (77–397) days and in 37 (54.4%) of 68 HBeAg-negative patients with median time of 215 (55–376) days. Among the patients with relapse, 8 (30.8%) HBeAg-positive patients had a biochemical relapse (ALT elevation>2×UNL) and 19 (51.4%) HBeAg-negative patients did. In multivariate analysis, age >40 years (OR, 10.959; 95% CI, 2.211–54.320; $P = 0.003$) and pretreatment HBV-DNA >2×10^6 IU/mL (OR, 9.285; 95% CI, 1.545–55.795; $P = 0.036$) were identified as independent risk factor for relapse in HBeAg-positive patients, and age >40 years (OR, 6.690; 95% CI, 1.314–34.057; $P = 0.022$) and HBcrAg level at end of treatment >3.7 log IU/mL (OR, 3.751; 95% CI, 1.187–11.856; $P = 0.024$) were selected in HBeAg-negative patients. In both groups, the potent of NA, duration of consolidation treatment, serum HBsAg, and IP-10 level at the time of discontinuation were not significantly related with relapse. During the study period, no liver decompensation or liver related death was reported. After relapse, HBV-DNA suppression was achieved in all patients who were re-treated by NA in both groups.
Conclusions: Our data suggested that clinical outcome of HBeAg-positive and negative CHB was similar after stopping antiviral agents, suggesting that discontinuation of NA might be considered carefully considering individual risk factors. Especially, old age and HBcrAg level at end of treatment could be useful predictor for relapse after in HBeAg negative patients.

Table (abstract P0650): Hazard ratios of events

Event(s)	NAs vs. No NAs					Nucleotide analogues vs. nucleoside analogues					Nucleotide analogues vs.no NAs		
	No. (%) of events		HR	95% CI	P-value	No. (%) of events		HR	95% CI	P-value	HR	95% CI	P-value
	NAs	No NAs				Nucleotides	Nucleosides						
Renal failure	100 (1.4)	270 (0.6)	0.91	0.69–1.21	0.517	6 (0.9)	94 (1.5)	0.58	0.25–1.34	0.202	0.48	0.21–1.12	0.091
RRT	49 (0.7)	96 (0.2)	1.31	0.85–2.03	0.225	3 (0.4)	46 (0.7)	0.62	0.19–2.05	0.433	0.70	0.22–2.28	0.558
Hip fracture	17 (0.2)	48 (0.1)	0.95	0.46–1.97	0.887	6 (0.9)	11 (0.2)	5.69	1.98–16.39	**0.001**	3.84	1.26–11.70	**0.018**
Spine fracture	15 (0.2)	53 (0.1)	0.79	0.42–1.49	0.469	1 (0.1)	14 (0.2)	0.79	0.09–6.53	0.823	0.62	0.09–4.41	0.631
All fracture	95 (1.3)	318 (0.7)	0.87	0.66–1.15	0.338	14 (2.0)	81 (1.3)	1.44	0.81–2.58	0.217	1.28	0.72–2.28	0.393

CI, confidence interval; HR, hazard ratio; NAs, nucleos(t)ide analogue; RRT, renal replacement therapy.

P0652

EFFECT OF TENOFOVIR ON KEY HEPATITIS B VIRUS MARKERS IN PREGNANT WOMEN DURING THE THIRD TRIMESTER AND POSTPARTUM

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Background and Aims: The risk of hepatitis B virus (HBV) transmission from mothers to babies has been well documented, particularly when the maternal viral load (VL) is high. The use of Tenofovir Disoproxil Fumarate (TDF) during the last trimester of pregnancy has been shown to reduce HBV load and significantly decrease the risk of perinatal transmission. The aim of this study was to assess the effect of TDF on key HBV markers in pregnant women with high VL in the third trimester of pregnancy.
Methods: 38 mothers were treated with TDF in the last trimester of pregnancy until birth or 12 weeks postpartum at the Liverpool Hospital, Sydney, Australia. A total of 119 blood samples were collected (at 3 time points if possible): T1 pre-treatment, T2 at birth, T3 around 12 weeks postpartum. They were tested for VL, quantitative (q) HBeAg and HBsAg, and the HBV reverse transcriptase (RT) region was analysed for drug resistance and surface antigen changes by population-based sequencing. 19 samples were also sequenced by next generation sequencing (NGS).
Results: The mean VL for all T1 samples was 7.98 $\log_{10}$ IU/mL, and the mean VL decrease from T1 to T2 was 3.36 $\log_{10}$ IU/mL. The mean qHBeAg also decreased: T1 (2206 IU/mL), T2 (1750 IU/mL) and T3 (1503 IU/mL). Two mothers were HBeAg negative at T1 and 3 became negative during treatment. However, no decrease was observed in the mean qHBsAg at T1, T2 and T3. All isolates were found to have wildtype sequences by population-based sequencing. Although NGS analysis detected several HBV RT amino acid changes at low frequencies in the mothers' samples, none are associated with drug resistance.
One baby was diagnosed with HBV at age 9 months. The mother's VL at birth was 4.42 $\log_{10}$ IU/mL, and the viral sequences from both the mother and baby were identical by population-based sequencing. In addition, the viral population profiles between the mother and baby samples were very similar by NGS analysis.
Conclusions: TDF remains the drug of choice for preventing the transmission of HBV from mother to baby despite the transmission case documented here. The mothers' HBV loads were dramatically reduced on treatment. Even after a short duration of therapy there was a decline in HBeAg, and no emergence of drug resistance mutations as seen with Lamivudine therapy. The transmission case observed is likely to be an in-utero transmission, given treatment compliance, a dramatic drop in viral load and administration of HBIG and HBV vaccination.

P0653

PERFORMANCE EVALUATION OF THE UPDATED ELECSYS ANTI-HBs II IMMUNOASSAY

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Background and Aims: Anti-HBs tests are employed worldwide to confirm a successful vaccination or to determine the stage of a Hepatitis B infection. The Elecsys Anti-HBs II test (Roche Diagnostics) has been modified to be compatible with all cobas e systems. In addition, one native antigen used in the test has been replaced by a recombinant antigen.
The aim of the study was to evaluate the specificity and sensitivity of the modified Elecsys Anti-HBs II immunoassay in blood donations, routine samples and clinically well-defined sera from individuals who have been vaccinated against Hepatitis B, or individuals who have recovered from a Hepatitis B infection. In addition, the modified assay was compared to other commercially available anti-HBs tests, and various dilutions from the 2nd WHO Standard were tested and compared between different tests.
Methods: The commercially available tests used in the study were as follows: Elecsys Anti-HBs and Anti-HBs II (Roche Diagnostics), Architect Anti-HBs (Abbott), Centaur Anti-HBs (Siemens), Liaison Anti-HBs (Diasorin). The WHO Standard dilutions were 0, 5, 10, 99, 250, 500, 750, 1000 and 1500 IU/L. 4088 samples from blood donations, 2017 samples from routine testing, 305 samples from vaccinated individuals and 270 samples from patients who recovered from Hepatitis B were investigated. External evaluation was performed at two different blood banks and two different routine laboratories. In addition, external routine samples were measured at the manufacturer (Roche Diagnostics).
Results: The specificity of the Elecsys Anti-HBs II in negative samples from blood donors at both blood banks was >99% and in negative samples from routine samples >98%. Sensitivity in vaccinated individuals and in patients who have recovered from Hepatitis B was 100%. The 10 IU/L reference sample from the WHO standards was measured by Elecsys Anti-HBs II and Architect anti-HBs to a high level of accuracy (10.1 IU/L and 9.9 IU/L, respectively). The 10 IU/L sample was overestimated by Elecsys Anti-HBs (11.0 IU/L), by Centaur Anti-HBs (12.1 IU/L) and by Liaison Anti-HBs (13.4 IU/L). Although the Liaison Anti-HBs overestimated the 10 IU/L standard, this test gave the best results over the entire range of WHO standard dilutions. The Architect Anti-HBs underestimated the concentration at the higher concentrations (>30%).
Conclusions: Finally, the Elecsys Anti-HBs II test exhibited a high agreement rate (98.5%) with Elecsys Anti-HBs and is a suitable replacement for the latter test on the cobas e system.

P0654

LONG-TERM SUSTAINED SUPPRESSION OF VIRAL REPLICATION IS ASSOCIATED WITH LOW HBsAg LEVELS IN PATIENTS WITH CHRONIC HEPATITIS B (CHB) TREATED WITH NUCLEOS(T)IDE ANALOGUES (NUCS)

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Background and Aims: The ideal endpoint of CHB treatment is loss of HBsAg but this is rarely achieved in patients treated with NUCs. The aims of the study were to assess long-term HBsAg levels in patients treated with NUCs and to identify factors associated with low HBsAg levels.
Methods: The study population consisted of 224 patients treated with NUCs for at least 1 yr. HBV-DNA levels were assessed by a TR-PCR assay and HBsAg levels by an ELISA quantitative method. Low HBsAg levels were defined as HBsAg <1000 IU/mL. Sustained virological response time (SVRT) was defined as the time elapsed between confirmed negativization of HBV-DNA and the last available HBV-DNA result.
Results: 70% of patients were male with mean age of 44.7±12 yrs; most of them had genotype D (59%) or A (30%), 28% were HBeAg+ and 22% had cirrhosis at baseline. Mean pre-treatment HBV-DNA levels were 6.08±1.47 log IU/mL and mean baseline ALT levels were 129±159 U/mL. First line of treatment consisted of first generation NUCs in 50% of the cases and second generation NUCs in the

remaining 50%. Median duration of treatment and SVRT were 6 yrs (range:1–15 yrs) and 4.6 yrs (range:0–12.6 yrs), respectively. 31% patients developed resistance to some NUC during treatment. At the last follow-up, HBV-DNA was negative in 97% of the patients and median HBsAg levels were 1606 IU/mL (range:1–21000 IU/mL). Last HBsAg levels were <1000 IU/mL and <100 IU/mL in 40.2% and 12.5% of the patients, respectively. Mean HBsAg levels were 4268±4293 IU/mL in patients treated <5 yrs, 2640±3244 IU/mL in those treated 5–10 yrs, and 1480±2217 IU/mL in those treated >10 yrs ($p = 0.007$ between <5 yrs and 5–10 yrs; $p = 0.01$ between 5–10 yrs and >10 yrs). Variables associated with low HBsAg levels in the univariate analysis: male sex ($p = 0.001$), development of NUC resistance ($p = 0.01$), baseline HBV-DNA <5.8 log IU/mL ($p = 0.01$), treatment for more than 5 yrs ($p < 0.001$), and SVRT >4 yrs ($p < 0.001$). Male sex (OR 2.06, 95% CI: 1.05–4.02, $p = 0.01$), baseline HBV-DNA <5.8 log IU/mL (OR 2.06, 95% CI: 1.05–4.02, $p = 0.01$), and SVRT >4 yrs (OR 2.91, 95% CI: 1.43–5.89, $p = 0.01$) were independently associated with low HBsAg levels in the multivariate analysis.

Conclusions: Long-term treatment with NUCs achieves suppression of viral replication in nearly all patients with CHB and low HBsAg levels in a significant proportion of them. This sustained suppression of viral replication seems to be playing a significant role in obtaining low HBsAg levels with its potential clinical implications.

P0655
LAMIVUDINE PROPHYLAXIS PREVENTS HEPATITIS B REACTIVATION IN HBsAg-NEGATIVE/ANTI-HBc-POSITIVE PATIENTS UNDERGOING RITUXIMAB-BASED CHEMOTHERAPY FOR NON-HODGKIN'S B CELL LYMPHOMA

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Background and Aims: Hepatitis B surface antigen (HBsAg)-negative/anti-hepatitis B core antigen (anti-HBc)-positive patients undergoing Rituximab (RTX)-based chemotherapy (R-CT) for non-Hodgkin's B cell lymphoma (NHL) face up to 27% risk of hepatitis B virus (HBV) reactivation. Aim of the study was to assess the efficacy and safety of lamivudine (LMV) prophylaxis in these patients.

Methods: Sixty-seven consecutive HBsAg-negative/anti-HBc-positive patients (median age 70 yrs, 61% males, 13% HCV seropositive, 100% serum HBV DNA negative by sensitive PCR assay, 75% anti-HBs positive, 100% with ALT <ULN) undergoing different R-CT protocols (48% R-CHOP) in two hematological centres were retrospectively enrolled. LMV prophylaxis was started before the first R-CT dose and planned to last for 18 months after the end of R-CT. R-CT was administered for 6 cycles (range: 1–15) during 5 (range: 1–38) months, while LMV was administered for 20 (range: 3–54) months. Serum ALT, HBsAg and HBV DNA levels by PCR assay were assessed every 3–4 months.

Results: All patients remained HBV DNA and HBsAg negative during a median of 20 months (range: 2–60), including both the LMV prophylaxis and the subsequent off treatment follow-up. Anti-HBs titers declined in 18% of patients but none lost serum reactivity for anti-HBs. Overall, 2 patients had an increase of ALT (>ULN) but this event was unrelated to HBV reactivation and 7 patients died of liver unrelated causes. No safety issues related to LMV were recorded.

Conclusions: LMV monotherapy is an inexpensive, safe and effective prophylaxis regimen to prevent HBV reactivation in such a high-risk population as HBsAg-negative/anti-HBc-positive patients receiving RTX-based chemotherapy for non-Hodgkin's B cell lymphoma.

P0656
ADD ON PEGINTERFERON TO ADEFOVIR ENHANCES HBsAg LOSS IN INACTIVE HBV CARRIERS

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Background and Aims: HBsAg loss, considered to be the ideal outcome of HBV infection, occurs spontaneously at low rate in inactive HBV carriers not candidates for therapy. We investigated the ability of treatment to achieve accelerate incidence of HBsAg loss in inactive carriers

Methods: 91 inactive carriers from a well phenotyped cohort (Journal of Clinical Virology 2013; 58: 401) were followed for a 2 years period. At that time point 69 remained untreated, 22 underwent 4 years adefovir therapy. At 4 years 16 patients received 48 weeks peginterferon, 6 continued adefovir monotherapy. All the patients ended therapy at 5 years, and were followed for at least 2 years after treatment cessation.

Results: At baseline; ALT were 24±7 and 23±7 (ns), HBV genotypes A, B–C, D, E observed in 33%, 15%, 33%, 19% in untreated and 37%, 20%, 37%, 6% in treated patients (ns). HBsAg levels 3.24±1.0 and 3.34±0.92 log IU/ml (ns), HBV DNA levels 2.57±0.97 and 2.90±0.84 log IU/ml (ns), in untreated and treated patients, respectively. At the end of follow up, HBV DNA was undetectable in 12/69 (17%) of untreated and 9/22 (41%) of treated patients ($p < 0.02$), an HBsAg loss was observed in 10/69 (15%) of untreated and 7/22 (32%) of treated patients, ($p = 0.06$). A baseline HBsAg level >3.3 log IU/ml shows a negative predictive value (NPV) of 92% for HBs loss. Among the treated patients a HBs loss was observed in 0/6 (0%) receiving adefovir and 7/16 (44%) receiving add-on peginterferon ($p = 0.04$). Baseline HBsAg levels were 3.50±0.99 and 2.88±1.16 log IU/ml in adefovir and add-on peginterferon therapy patients, respectively (ns). In the 16 patients receiving add-on peginterferon therapy, baseline HBsAg levels were 1.97±0.83 and 3.59±0.74 log IU/ml in patients with or without HBsAg loss, respectively ($p < 0.001$). An HBsAg titer >3.3 log IU/ml shows a NPV 100% for HBsAg loss. No patient relapsed.

Conclusions: In inactive HBV carriers treated with adefovir add-on peginterferon dramatically accelerates the HBsAg decline and rate of HBsAg loss (44%), in comparison to untreated patients (17%).

P0657
ADDING TENOFOVIR TO PEGYLATED INTERFERON ENHANCES END OF TREATMENT HBsAg LOSS IN HBeAg NEGATIVE CHRONIC HEPATITIS B PATIENTS

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Background and Aims: It remains unclear whether adding a nucleot(s)ide analogue enhances the efficacy of pegylated interferon alfa-2a (PegIFN) by accelerating HBsAg decline and clearance. We aimed to assess efficacy of the combination of PegIFN alfa-2a + tenofovir (TDF).

Methods: 88 CHB patients [26 HBeAg(+); 62 HBeAg(−)] treated 48 weeks. HBsAg measured at baseline, weeks 12, 24, end of therapy (EOT), 24 weeks after treatment cessation.
Results: 43 patients [13 HBeAg(+); 30 HBeAg(−)] received PegIFN monotherapy and 45 patients [13 HBeAg(+); 32 HBeAg(−)] received the combination of PegIFN +TDF. SVR was 38%, 23% and 45% in the overall population, HBeAg(+) and HBeAg(−), respectively. HBe(+) positive patients were not further analysed.
Among the 62 HBe(−) patients, EOT response was 90% and 100% in PegIFN and PegIFN +TDF patients, respectively. SVR was observed in 33% and 53% of PegIFN and PegIFN +TDF patients, respectively. EOT HBsAg loss was observed in 6.6% and 12.5% of PegIFN and PegIFN +TDF patients, respectively. The negative predictive value (NPV) of baseline HBsAg >1500 IU/ml for SVR and HBs loss were 81% and 86% in PegIFN patients and 60% and 95% PegIFN +TDF patients.
The NPV of week 24 HBsAg decrease <0.5 log IU/ml were 73% and 57% of PegIFN alfa-2a and PegIFN +TDF patients, respectively. An end of follow-up HBs loss was observed in 20% and 19% of PegIFN and PegIFN +TDF patients, respectively.
Conclusions: In patients receiving PegIFN +TDF experience higher SVR rate 53% versus 33% and EOT HBsAg loss 12.5% versus 6.6% than patients receiving PegIFN monotherapy.

P0658
A NEW PROGNOSTIC MODEL FOR 1-YEAR MORTALITY IN PATIENTS WITH DECOMPENSATED HEPATITIS B VIRUS-RELATED LIVER CIRRHOSIS RECEIVING ANTIVIRAL TREATMENT

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Background and Aims: Considering the remarkable survival gain achieved with the use of oral antiviral agents, we aimed to develop a more efficient prognostic model to predict 1-year mortality after initiating antiviral treatment in patients with decompensated hepatitis B virus (HBV)-related cirrhosis.
Methods: We investigated 194 treatment-naive decompensated cirrhotic patients who were administered lamivudine or entecavir for a median of 41 months. Prediction models A (pretreatment) and B (on-treatment) were created by multivariate Cox-regression analysis based on baseline and 1-month follow-up data, respectively.
Results: At baseline, 75.8% of patients had ascites, and the median Child-Turcotte-Pugh (CTP) and model for end stage liver disease (MELD) score was 9 and 13.8, respectively. Twenty-seven events (24 deaths + 3 liver transplants [LTs]) occurred within the first year of treatment, accounting for 52% of all events during follow-up. Models A and B consisted of conventional risk factors categorized differently from traditional prediction models. The area under the receiver operating characteristic curve for predicting 1-year events at baseline was significantly higher for Model A than for CTP score, MELD score, and Fontana index (0.953 vs. 0.902, 0.861, and 0.851, respectively; all $P < 0.05$). Furthermore, pretreatment Model A scores in conjunction with Model B scores within 6 months of follow-up could predict 89% (24 of 27) of 1-year events with 100% specificity; this rate was markedly higher than the 41% (11/27) for CTP score, 63% (17/27) for MELD score, and 26% (7/27) for Fontana index.
Conclusions: Our new prediction models will help to determine the need for LT in patients with decompensated HBV-related cirrhosis before and during antiviral treatment.

P0659
CYTOKINE RESPONSES IN CHRONIC HEPATITIS B PATIENTS DOSED WITH THE NUCLEIC-ACID POLYMER REP2139-Ca

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Background and Aims: Current treatment regimens for patients with chronic hepatitis B (CHB) are only successful in eradicating HBsAg (functional cure) in a minority of patients. Treatment with the HBsAg release inhibitor REP2139-Ca may be a promising new treatment option for CHB patients. The clearance of HBsAg could be the key to restoring the immune response against the hepatitis B virus. Immunologic activity in response to treatment with REP2139-Ca was analysed and patients who responded to therapy were compared to those who did not.
Methods: 12 Patients with HBeAg positive CHB (mean HBVDNA 10log7.36 IU/ml) participating in a phase 2 study were dosed with the nucleic-acid based amphipathic polymer REP2139-Ca for 20–38 weeks. Responders to REP2139 (defined as clearance of serum HBsAg) were subsequently treated with an add-on immunomodulatory agent (peginterferon alpha-2a and/or thymosin alpha-1). Serum samples were collected at baseline, during REP2139-Ca treatment (week 5, 12, 24), during add-on immunomodulatory treatment and follow-up (12–39 weeks after cessation of therapy). Cytokine and chemokine levels were measured using a Luminex 27-plex immunoassay (Affymetrix eBioscience, San Diego, USA).
Results: 9/12 patients cleared HBsAg during dosing with REP2139-Ca. From all cytokines analysed, IFNγ, TNFα, IL-1α, IP-10 and IL-18 significantly increased during treatment with REP2139-Ca. IL-7 and IL-8 serum concentration decreased. Add-on immunomodulatory treatment did not add to this effect as there were no significant differences at week 24 of REP2139-Ca treatment and during immunomodulatory treatment. IL-6, IL1RA and IL-10 did not show significant changes at any of the time points measured. Although the number of patients in this observational study was limited, no significant differences were observed in cytokine levels between responders and non-responders to REP2139-Ca.
Conclusions: In patients treated with REP2139-Ca, significant changes were observed in serum chemokine and cytokine levels during therapy compared to baseline. These results suggest that upon treatment with REP2139-Ca, immune-responses are remodeled, possibly restoring antiviral activity against hepatitis B.

P0660
A SPECIFIC KIR/HLA-C GENOTYPE IS ASSOCIATED WITH IMMUNE ACTIVITY AND RESPONSE TO PEGINTERFERON AND ADEFOVIR IN HBeAg-POSITIVE CHRONIC HEPATITIS B PATIENTS

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Background and Aims: The antiviral activity of NK cells partially depends on the interaction of killer cell immunoglobulin-like receptors (KIR) and their HLA-C ligands. Genetic variations in KIR/HLA-C influence the outcome of acute hepatitis C infection and single nucleotide polymorphisms (SNPs) in HLA-C have been implicated in hepatitis B chronicity. However, the role of these variations in relation to treatment outcome in chronic hepatitis B (CHB) patients is unknown.

Methods: 86 CHB patients (41 HBeAg-positive; 45 HBeAg-negative) who completed 48 weeks of peginterferon alfa-2a and adefovir combination therapy followed by a treatment-free follow-up (week 72) were included. Patient DNA was isolated from PBMC. SNPs located within or near (±500 base pairs) the HLA-C gene were genotyped with the Illumina Human Omni1-Quad BeadChip (n = 12). Genotyping of KIR2DL1, KIR2DL2 and KIR2DL3 was performed by PCR. Combined response at week 72 (HBeAg negativity, HBV-DNA ≤2,000 IU/mL, and ALT normalization) was achieved in 14/41 HBeAg-positive and 17/45 HBeAg-negative patients.
Results: One SNP in HLA-C (rs2308557; A allele) was significantly associated with combined response in HBeAg-positive patients after correction for multiple testing (p = 0.003), but not in HBeAg-negative patients. This SNP is linked to the presence of an Asn or Lys at position 104 of HLA-C, important for binding specific KIRs (HLA-C group C1 or C2, respectively).
The distribution of C1C1, C1C2, C2C2 genotypes in HBeAg-positive patients was 7 (17%), 17 (42%), and 17 (42%), respectively. HBeAg-positive patients with the C2 allele had higher rates of combined response (13/24 vs 1/17, p = 0.001), and higher baseline ALT levels (median ALT 136 vs 50 U/L, p = 0.002) than patients with only C1 alleles. The C2 allele is specifically recognized by the KIR2DL1 receptor, which was present in all patients. Presence of HLA-C2/KIR2DL1 predicted response independent of HBV genotype A, HBV-DNA and ALT levels in multivariable analysis (p = 0.007). In contrast, the HLA-C1C1/KIR2DL3 combination was more prevalent in HBeAg-positive non-responders than combined responders (15/27 vs 1/14, p = 0.003).
Conclusions: A specific KIR/HLA-C combination was strongly associated with combined response in HBeAg-positive patients. These findings support an important role for the interaction of NK cell receptors and their HLA-C ligands in determining the host immune activity and response to interferon-based therapy in chronic hepatitis B patients.

P0661

PREDICTION OF HBeAg SEROCONVERSION IN HBeAg-POSITIVE CHRONIC HEPATITIS B PATIENTS TREATED WITH ENTECAVIR USING ALT AND PLATELET COUNT: RESULTS FROM A LARGE EUROPEAN MULTI-CENTER STUDY

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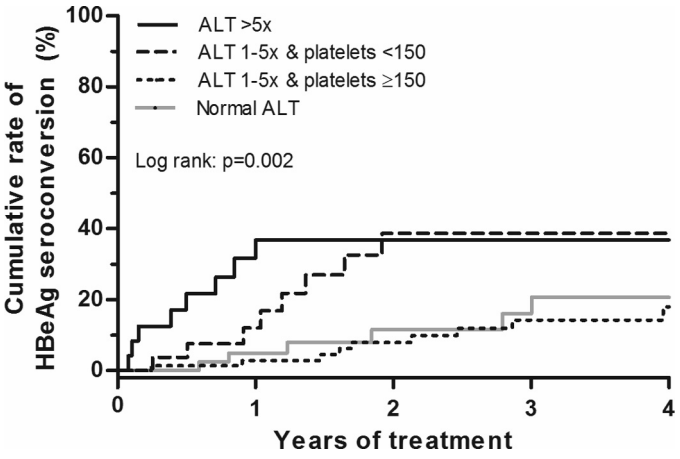
Background and Aims: For HBeAg-positive chronic hepatitis B (CHB) patients treated with nucleos(t)ide analogue (NA) therapy, HBeAg seroconversion is an important endpoint as guidelines suggest the possibility to discontinue NA therapy after HBeAg seroconversion. However, the HBeAg seroconversion rate is suboptimal with current first-line NA and there are no reliable tools to predict HBeAg seroconversion in these patients.
Methods: This is a large European multi-center cohort study (VIRGIL) enrolling all consecutive CHB mono-infected patients treated with entecavir (ETV) monotherapy for at least 3 months. Further eligibility criteria were: HBeAg positivity and HBV DNA >2,000 IU/mL at the initiation of ETV. Patients were prospectively monitored at least every 3 months. HBeAg seroconversion was defined as loss of HBeAg and the appearance of anti-HBe confirmed in a consecutive sample.
Results: In total, 218 HBeAg-positive patients were eligible for analysis. Median treatment duration was 2.9 years (IQR 1.5–4.1). Baseline characteristics were: median age 36 years, 75% male, 45% Caucasian, mean HBV DNA 7.2 log IU/mL and median ALT 1.9× upper limit of normal (ULN). ETV was effective in the suppression of viral replication resulting in virological response (HBV DNA <75 IU/mL) of 49% at 1 year, 75% at 2 years and 94% at 4 years. HBeAg seroconversion was confirmed in 41 patients resulting in cumulative HBeAg seroconversion rates of 9% at 1 year, 17% at 2 years and 24% at 4 years. Only baseline ALT levels and platelet count were associated with HBeAg seroconversion. Patients with ALT >5× were more likely to achieve HBeAg seroconversion than patients with ALT 1–5× (hazard ratio [HR] 2.7; p = 0.017), or normal ALT (HR 3.5; p = 0.021). In addition, a platelet count of <150×10^9/L was associated with an increased chance of HBeAg seroconversion, but only among patients with ALT 1–5× (HR 3.5; p = 0.01). Therefore, we combined the ALT level and platelet count to optimize the prediction of HBeAg seroconversion (Figure).
Conclusions: In this large real-life cohort of HBeAg-positive patients treated with ETV, the HBeAg seroconversion rate is low. For these patients, we present an easy to use model to predict HBeAg seroconversion using baseline ALT level and platelet count which are readily available objective parameters. Patients with a low chance to seroconvert should be consulted regarding long-term NA therapy or peginterferon addition strategy.



P0662

24 WEEKS OF PEGINTERFERON MAY BE AS GOOD AS 48 WEEKS IN HBeAg-POSITIVE GENOTYPE B PATIENTS WITH AN EARLY HBsAg RESPONSE: POOLED ANALYSIS OF TWO RANDOMIZED TRIALS

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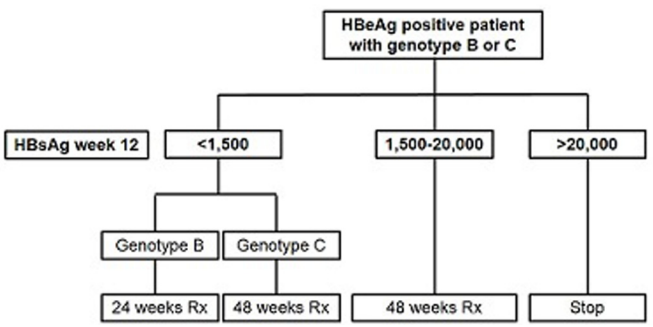
Background and Aims: Early HBsAg decrease during peginterferon (PEG-IFN) therapy is associated with high rates of sustained response. We hypothesized that 24 weeks (wks) may be as good as 48 wks in patients with low HBsAg levels at wk 12.
Methods: HBeAg-positive patients treated with PEG-IFN alfa-2a (180 mcg/wk) or alfa-2b (1.5 mcg/kg/wk) for 24 or 48 wks in two randomized trials (Neptune and P05170) were analysed. HBsAg at wk 12 was defined as low (<1,500 IU/mL), intermediate (1,500–20,000) or high (>20,000). Response was defined as HBeAg loss with HBV DNA <2,000 IU/mL at 24 wks off-treatment.
Results: 593 patients were analysed; 292 (49%) treated for 24 weeks, 301 (51%) for 48 weeks. HBV genotypes were A/B/C/D/other in 10/245/317/12/9. In the overall population, response was achieved more frequently with 48 weeks than with 24 weeks of therapy (21% vs 13%, p = 0.008). HBsAg levels at wk 12 were low in 148 (25%), intermediate in 298 (50%) and high in 147 (25%) with comparable rates among the treatment groups (p = 0.890). Among patients with high HBsAg levels, response rates were <5% regardless of therapy duration. Among patients with intermediate HBsAg levels, 48 wks was superior to 24 wks (23% vs 9.7%, p = 0.002). Conversely, among patients with low HBsAg levels, response rates were similar for patients treated with 24 vs 48 wks (31% vs 34%, p = 0.642). Stratification by HBV genotype (B or C only) showed that among patients with low HBsAg levels, response rates with 24 vs 48 wks of PEG-IFN were similar for genotype B [numerically higher with 24 wks, 46% (17/37) vs 31% (11/35), p = 0.207], but not for genotype C [higher with 48 wks, 14% (5/36) vs 38% (14/37), p = 0.02]. Among patients with intermediate HBsAg levels at week 12, 48 weeks of therapy was superior to 24 weeks regardless of HBV genotype. All findings were homogenous across the two separate cohorts. Based on these findings a new algorithm could be proposed for management of HBeAg-positive patients treated with PEG-IFN (figure) dependent upon further validation.



Conclusions: Genotype B patients with low HBsAg levels at wk 12 may potentially be able to shorten therapy to 24 wks, allowing a reduced therapy duration in about one-third of genotype B patients. Genotype B and C patients with intermediate HBsAg levels benefit from a full 48 wk course, as do genotype C patients with low HBsAg levels. All patients with high HBsAg levels at wk 12 have low response rates regardless of treatment duration.

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FACTORS ASSOCIATED WITH NON-ADHERENCE TO ANTIVIRAL TREATMENT FOR HEPATITIS B

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Background and Aims: Anti viral therapy for chronic hepatitis B (CHB) is effective and reduces the risk of cancer and cirrhosis but is often for an indefinite duration. Adherence is important to prevent the development of resistance to current therapies and prevent complications.
Adherence can be measured by pharmacy adherence measures. The medication possession ratio (MPR) is less susceptible to social desirability bias than physician or self-report and is a measure of maximal adherence. The aim of this study was to measure adherence in public hospital patients over a 3-year period using MPR and look at factors associated with non-adherence.
Methods: A retrospective analysis of pharmacy records of patients dispensed antiviral therapy for CHB from 4 major hospitals in Melbourne between 2010–2103. De identified demographic information including age, sex, Indigenous status, country of birth, requirement for an interpreter, spoken language, postcode of residence were matched with hospital record number for 3 of the 4 sites.
The medication possession ratio (MPR) was the outcome measure. It was calculated from the amount of tablets dispensed from the hospital pharmacy over a time period and analysed as a continuous and dichotomous variable (<0.9 defined as non-adherent to therapy). Univariate analysis on factors affecting non-adherence was performed. Data were analysed in STATA 13 with two-sided tests of proportions or Chi-squared test.
Results: Records of 980 patients were included in the analysis. Median MPR for all patients was 0.99 inter quartile range 0.92–1.00. This did not vary significantly by hospital. Significant factors affecting non-adherence in univariate analysis, included patients aged <35 years (p = 0.002) or who required an interpreter (p = 0.0005). Socio-economic status as defined by postcode of residence (p = 0.38), gender (p = 0.46) and distance to hospital (p = 0.56) were not significant factors affecting adherence.
Conclusions: This is the largest study reporting on factors associated with adherence to hepatitis B treatment across multiple hospitals in Australia. While adherence was high, one in five patients were non-adherent. Extended analysis including additional demographic factors and multivariate analysis will be presented. This study will guide the development of strategies to improve adherence to treatment for people living with chronic hepatitis B.

Table 1: Adherence of hospital patients and univariate analysis of factors affecting non-adherence of patient receiving antiviral treatment for chronic hepatitis B

		Number of observations (Total)	(%)		
Adherence measure	MPR < 0.90	205 (980)	20.92		
	MPR ≥ 0.90	775 (980)	79.08		
Factors			MPR<0.90 (%)	MPR≥ 0.90 (%)	p-value
Age < 35 years	Yes	190 (977)	28.42	71.12	0.002
	No	787 (977)	19.06	80.94	
Gender	M	590 (869)	20.85	79.15	0.46
	F	279 (869)	21.85	78.85	
Interpreter required	Yes	174 (577)	32.18	67.82	0.0002
	No	403 (577)	19.60	80.40	
Socio-economic status					
Most disadvantaged	1	161 (575)	22.36	71.64	0.308
	2	93 (575)	29.03	70.97	
	3	95 (575)	21.05	78.95	
	4	160 (575)	20.00	80.00	

P0664

INVESTIGATION OF RESIDUALS RESPONSIBLE FOR BINDING OF TRIAZOLO-PYRIMIDINE INHIBITORS WITH HBsAg BASED ON MOLECULAR DOCKING AND PHARMACOPHORE METHODS FOR DESIGNING POTENT HBsAg INHIBITORS

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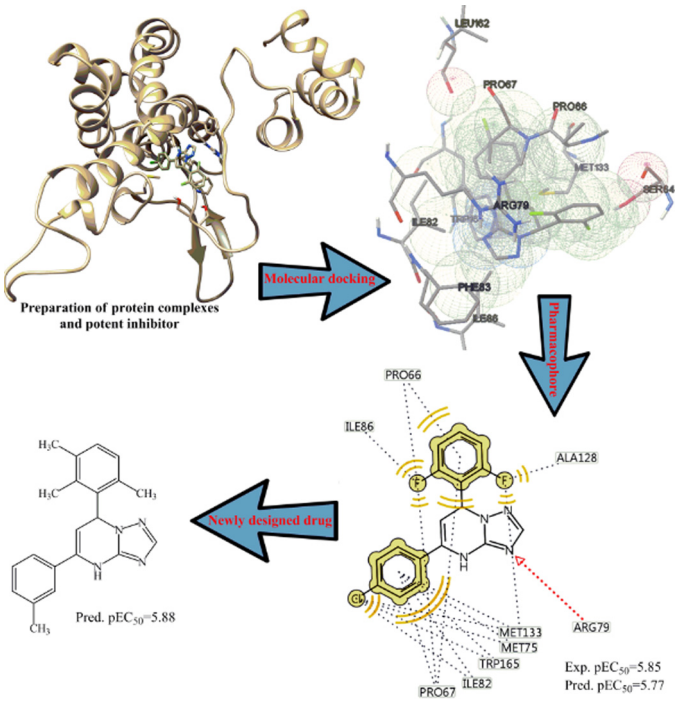
Background and Aims: The true knowledge of binding pockets of Hepatitis B virus surface antigen can provide valuable information regarding the better designing of novel HBsAg inhibitors, however due to less information about the residuals evolved in structure of HBsAg, it is a challenging work to perform the drug design simulation. The primary purpose of this work is to compare the selected residuals of HBsAg structure with QSAR modeling analyses, and then identifying the residuals responsible for interactions with drugs; Secondly, using these results for designing some potent inhibitors by docking simulation.
Methods: The data set and biological activities (EC_{50}) of triazolo-pyrimidine inhibitors were taken from literature [J. Med. Chem. 54 (2011) 5660–5670]. The protein complexes were selected from literature [Virology 370 (2008) 362–372], and then AutoDock 4.2 program was used to understand their existing interactions. The results of docking were then analyzed with LigandScout 3.03 program. After deriving the key features for increasing the inhibition activities, new compounds were designed and their activities were predicted using a validated QSAR model (GA-OLS-MLR).
Results: From QSAR analyses, it was observed that the most responsible molecular descriptors regarding the inhibition activities are distance between Cl atoms, molecular eccentric connectivity, and three-dimensional arrangement of the atoms. This is also observed that the atomic polarizabilities cause decrease in biological activities. The results are in line with molecular docking in which it was observed that the hydrophobic characteristics play crucial role in improvements of pEC_{50}. Pharmacophore and molecular docking resulted in identification of several residuals which are shown in Figure 1. Consequently, some novel drugs were designed and their biological activities were predicted using a robust QSAR model as follows:

pEC_{50} = −1.996(±1.781) − 24.13(±3.141)RBF − 1.284(±0.3572)BEHp8 + 15.46(±1.551)MEcc − 3.573(±0.3357)PJI3 + 0.0496(±0.0071)RDF070u − 3.374(±0.6840)HATS5u + 0.300(±0.0671)B08[Cl–Cl].

Several validation methods were used to evaluate the prediction capability of the developed model, and the obtained results confirmed that the built model can be used as prediction tool for subsequent novel drugs.
Conclusions: Based on the obtained results some new inhibitors targeting HBsAg were designed and then their 50% effective concentrations were predicted using the constructed model. The newly designed potent compound i shown in Figure 1.



P0665

HBV VARIANTS PRESENT IN TREATMENT NAÏVE PATIENTS CAN PREDICT RESPONSE TO NA THERAPY IN IMMUNE CLEARANCE DISEASE

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Background and Aims: GS-US-174-0103 is a phase 3 study that evaluated tenofovir (TDF) in HBeAg-positive chronic hepatitis B (CHB). Approximately 25% of patients underwent HBeAg seroconversion (SC) by W48, with an additional 25% by W192 (Year 4). Additionally, 11% of patients achieved HBsAg loss by W192. The aim of this study was to quantify clinically relevant mutations within regulatory and structural regions of the HBV genome using next generation sequencing (NGS) at baseline and correlate to these with treatment response to TDF.
Methods: Illumina MiSeq full genome NGS was performed on a subset of 94 baseline samples from patients enrolled in study 103 with longitudinal clinical data to W192. A specialised platform was developed to enable quantitative determination of SNPs and

insertion/deletion events across the whole genome. The results of NGS were correlated with virological and serological data including HBV DNA, HBsAg, and HBeAg levels as well as time to viral load undetectable, HBeAg SC and HBsAg loss.

Results: Among the 94 patients, the rate of HBeAg SC was 56% and HBsAg loss was 19% by W192. Using a threshold for detection of 1%, known HBV variants were detected in patients across regulatory (NRE, BCP, PC) and structural (core, PreS1/2, HBsAg, Pol) regions (Table 1). NGS was able to detect and quantify low frequency mutations that were not detected by population sequencing.

Table 1. Key mutations identified within the 103 cohort

	n (%)	
	Pop[n]	NGS
Negative Regulatory Element (NRE) G1613A	11 (12%)	31 (33%)
Basal Core Promoter (BCP) A1762T/G1764A	32 (34%)	40 (43%)
BCP T/C1858G	9 (9%)	11 (12%)
Precore (PC) G1896A	14 (15%)	39 (41%)
Core P130T	21 (22%)	39 (41%)
PreS1 M1	0	11 (12%)
PreS2 M1	8 (9%)	27 (29%)
HBsAg P120T/G145R	6 (6%)	23 (24%)
Pol A181T/M204V/M250I	2 (2%)	28 (30%)

HBeAg SC by W192 was independently associated with older age ($p = 0.007$), higher baseline ALT, higher baseline HBeAg and genotype A (model $p < 0.0001$; $R^2 = 0.14$). Presence/absence of mutations within the NRE, BCP, PC and core regions at baseline did not independently impact on HBeAg SC.

Baseline HBeAg and HBsAg levels, genotype, fibrosis and presence of wild type variants at A1762, G1764, and P130 were independently associated with HBsAg loss by W192 (model $p < 0.0001$; $R^2 = 0.30$). Most importantly, higher HBsAg levels at baseline were predictive of HBsAg loss ($p < 0.0001$). Low levels of S and/or Pol mutations at baseline did not predict for viraemia at W24 or W48 of treatment.

Conclusions: This study has identified novel virological and serological markers of treatment response in immune clearance CHB. Patients with high baseline HBeAg and HBsAg levels and WT variants within the BCP and core are most likely to achieve HBsAg loss during TDF treatment. Our study further highlights the value of NGS for detecting clinically relevant mutations which may be overlooked by traditional sequencing.

P0666

ANALYZING THE MUTATION PATTERN OF MULTI-DRUG RESISTANT HEPATITIS B VIRUS DURING ENTECAVIR RESCUE THERAPY

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Background and Aims: Analyzing the mutation pattern of multi-drug resistance is important in treatment of CHB. However, evolution pattern of multi-drug resistant in patients who received ETV rescue therapy has been rarely studied.

Methods: Eight CHB patients with LAM- and ADV-resistant mutations showing partial virological response to ETV and subsequent ETV plus ADV therapy were enrolled. Mutation pattern was investigated. Direct sequencing, multiplex restriction fragment mass polymorphism (RFMP), and clonal analysis were compared for the utility of affecting on clinical decision of antiviral therapy. The binding affinity of TDF was investigated by molecular modeling.

Results: Among 160 clones at baseline, wild type HBV was present in 38.8%, LAM-resistant mutations in 30.6%, and ADV-resistant mutations in 34.4%. LAM-resistant mutation increased to 67.5% at the end of ETV therapy, and increased more to 69.4% at 12 month of ETV plus ADV therapy. ETV-resistant mutations reached 46.3% at the end of ETV therapy, but slightly decreased to 38.8% at 12 month of ETV + ADV therapy. ADV-resistant mutation decreased to 3.1% at the end of ETV therapy, but slightly increased to 9.4% at 12 month of ETV + ADV therapy. Among 256 of candidate mutation sites of HBV reverse transcriptase (rt) polymerase, direct sequencing detected 14.1%, multiplex RFMP 27.7%, and clonal analysis 35.2% in mutations. All mutations detected by direct sequencing revealed identical results in the respective analyses by clonal analysis and multiplex RFMP. Among 28 mutations which revealed discordant results between clonal analysis and multiplex RFMP, clonal analysis detected 24 (85.7%) mutations which RFMP did not. However, none of the mutations exceeding 40% of total clones by clonal analysis were missed by multiplex RFMP. The binding affinities of TDF with rtM204V/I+rtA181T/V mutant or rtM204V/I+rtN236T mutant were both strong, but the latter showed minutely decreased affinity due to rtN236T.

Conclusions: The clonal evolution of multi-drug resistant HBV revealed the selection of LAM-resistant (±ETV-resistant) HBV during ETV rescue therapy, and the main reason for suboptimal response might be LAM-resistance (±ETV-resistance) which predominated over ADV resistance. Multiplex RFMP showed high sensitivity for detecting LAM-resistant (±ETV-resistant) mutation taking less time and labor than clonal analysis. The potent drug TDF showed strong binding affinity in multi-drug resistant HBV regardless of their co-location.

P0667

INTERRUPTION OF NUCLEOS(T)IDE ANALOGUE THERAPY FOR HBeAg-NEGATIVE CHRONIC HEPATITIS B – A NEW CONCEPT TO ACHIEVE HBsAg DECLINE?

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Background and Aims: HBsAg loss during treatment with nucleos(t)ide analogues (NA) is a rare event. Thus, treatment duration with NA is usually not finite and stopping rules before HBsAg loss are not clearly defined, especially in HBeAg-negative patients.

Methods: In a prospective pilot trial, we stopped NA treatment in 15 patients with HBeAg-negative chronic hepatitis B. Inclusion criteria were ongoing antiviral treatment with NA and suppressed HBV DNA <20 IU/ml for at least 3 years as well as HBsAg <3500 IU/ml. Patients with co-infection, other concomitant liver disease, liver cirrhosis and immunosuppressive therapy were excluded. All patients had an indication for antiviral treatment at the time point of NA initiation according to international guidelines. At every visit, patients were assessed for virological relapse, defined by HBV DNA >2000 IU/ml. Quantitative HBsAg levels one year before and after treatment cessation were compared to assess differences in HBsAg levels. Serum IP-10 (CXCL10) levels of 13 patients were analysed using multiplex technology (BioRad Bio-Plex-System).

Results: So far, complete data are available for 13 patients with one year follow-up (FU). Virological relapse after treatment cessation was detected in 4 patients (31%) at week 4, in 7 patients (54%) at week 8 and in 1 patient at week 24. No virological relapse occurred in 1 patient. Interestingly, IP-10 levels were significantly enhanced at week 8 after stop of NA therapy ($p = 0.028$). Two patients developed an ALT flare >5 ULN during FU but no patient showed signs of liver failure or significant increase

of bilirubin. In 11/12 patients with virological relapse treatment was re-initiated with either entecavir or tenofovir and HBV-DNA was successfully suppressed at week 48 follow-up. Importantly, longitudinal monitoring before/after cessation showed a significant decline in median HBsAg levels from stop of NA therapy to 48 week FU (p = 0.0012) while there was no change during NA therapy one year before treatment cessation.

Conclusions: Treatment cessation in non-cirrhotic patients with HBeAg-negative chronic hepatitis B is safe but leads to virological relapse in >90% until week 24. Stop of NA therapy was associated with an induction of serum IP-10 that may help to explain the effect on HBsAg decline. Thus interrupting NA treatment and inducing immune responses should be further investigated as an option to facilitate HBsAg loss.

P0668

ON-TREATMENT HBsAg KINETICS TO PREDICT LONG-TERM HDV RNA RESPONSE TO PEG-IFNa TREATMENT OF HEPATITIS DELTA

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Background and Aims: The only treatment option for hepatitis D (delta) is PEG-Interferon alfa (PEG-IFNa) leading to off-treatment HDV-RNA suppression in less than 25% of cases. HBsAg and HDV RNA levels correlate in untreated HDV-infected patients. In HBV monoinfection, stopping rules based on HBsAg levels or declines have been established for PEG-IFNa-based therapies. The role of on-treatment HBsAg kinetics during PEG-IFNa treatment of hepatitis D (delta) virus (HDV) infection is unknown.
Consequently the primary aim is to investigate if HBsAg kinetics during PEG-IFNa therapy can predict HDV RNA response 24 weeks after therapy.

Methods: 120 HDV-RNA-positive patients were treated for 96 weeks with PEG-IFNa-2a in combination with tenofovir or placebo in the HIDIT-2 study (Wedemeyer et al. EASL 2014). Here we selected all patients who received at least 80 weeks of therapy and for whom HDV-RNA-values 24 weeks after the end of therapy were available (n = 93). HBsAg was determined during treatment weeks 12, 24, 36 and 48.

Results: HDV RNA was undetectable at post-treatment week 24 (week 120; PTw24 response) in 31 of the 93 patients (33.3%). Mean quantitative HBsAg levels did not decline before week 24 (−0.21 log IU/ml) but gradually decreased thereafter (week 36 −0.31 log IU/ml; week 48 −0.46 log IU/ml). Mean HBsAg levels did not differ between off-treatment HDV RNA responder patients, off-treatment HDV RNA relapser and HDV RNA non-responder patients. HBsAg declines of more than 1 log IU/ml from week 24 onwards were highly predictive of long-term HDV RNA suppression with PPVs between 81% and 100%. However, only few patients achieved an HBsAg decline of >1 log IU/ml during treatment (5, 10 and 16 patients at treatment weeks 24, 36 and 48, respectively with PPVs for PTw24 response of 100%, 90% and 81%). Patients who did not show any HBsAg decline at treatment week 24 (n = 32) had a low chance for achieving a PTw24 HDV RNA response (12.5%; NPV 87.5%). However, we failed to identify distinct HBsAg cut-offs with negative-predictive values of >90%.

Conclusions: HBsAg kinetics during the first year therapy are associated with off-treatment HDV RNA response after 2 years of PEG-IFNa-based treatment of hepatitis D (delta). However, reliable stopping rules based on quantitative HBsAg levels to avoid unnecessary prolonged PEG-IFNa therapy could not be established.

P0669

CONSOLIDATION THERAPY WITH ENTECAVIR CAN PREVENT POST-TREATMENT HBsAg REBOUND IN HBeAg-POSITIVE CHRONIC HEPATITIS B PATIENTS TREATED WITH PEGINTERFERON ALPHA

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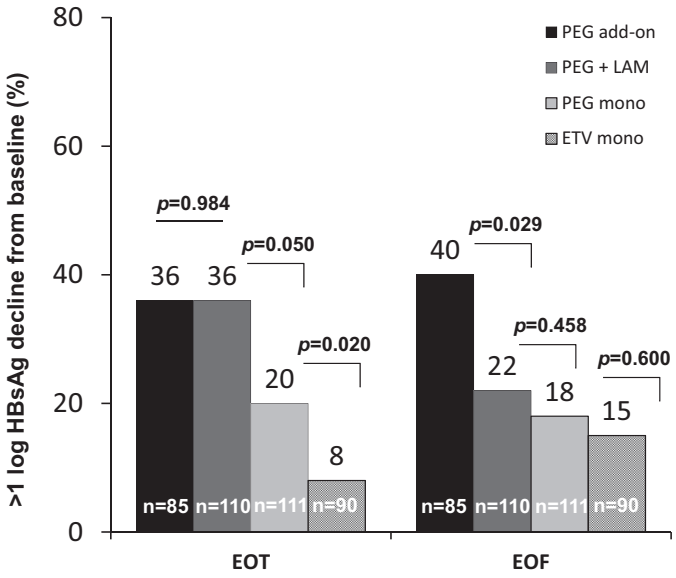
Background and Aims: It is unknown whether adding-on peginterferon (PEG-IFN) to entecavir (ETV) leads to more HBsAg decline compared to PEG-IFN monotherapy or de novo combination therapy, and whether consolidation therapy prevents HBsAg relapse after PEG-IFN cessation.

Methods: We performed a post-hoc comparison of 396 HBeAg-positive patients treated for 72 weeks with ETV + 24 weeks PEG-IFN add-on from week 24–48 (add-on, n = 85), 72 weeks with ETV monotherapy (n = 90), 52 weeks with PEG-IFN monotherapy (n = 111) and 52 weeks PEG-IFN + LAM (combination, n = 110) within 2 international randomized trials (ARES and HBV 9901 respectively). The extent of HBsAg decline was assessed on-treatment, at end-of PEG-IFN (EOT) and 6 months after PEG-IFN (EOF). Differences in baseline characteristics were accounted for using propensity scores as inversed weighting in regression analysis.

Results: Of 396 patients, 75% was male, mean age was 33 years and HBV genotype A/B/C/D was present in 22%/13%/27%/36% respectively. For add-on, combination, PEG-IFN mono and ETV the mean estimated HBsAg level decline achieved at EOT was −0.80, −1.27, −0.77 and −0.32 log IU/mL (combination vs add-on p = 0.045) and −0.84, −0.81, −0.68, and −0.33 log IU/mL, at EOF respectively (p > 0.05 for PEG-IFN arms). The estimated proportion of patients with ≥1 $\log_{10}$ HBsAg reduction from baseline at EOT and EOF was 36% and 40% for add-on, 36% and 22% for combination, 20% and 18% for PEG-IFN mono and 8% and 15% for ETV, respectively (p = 0.984 and p = 0.029 for add-on vs combination at EOT and EOF, figure). Patients treated with PEG-IFN + NUC more frequently achieved a HBsAg decline ≥1 $\log_{10}$ at one year (36% vs 20%), but this advantage was only sustained in patients who continued ETV therapy until 6 months post PEG-IFN discontinuation: the estimated proportion of patients who relapsed from >1 at EOT to <1 $\log_{10}$ HBsAg reduction at EOF was 5%, 46%, 38% and 0%, respectively. Estimated HBeAg loss rates at EOT were 22%, 40%, 27% and 11% (p = 0.039 for add-on vs combination) and 35%, 31%, 33% and 23% at EOF (p > 0.05 for PEG-IFN arms).

Conclusions: The combination of PEG-IFN with a NUC either as de novo for 52 weeks or as add-on for 24 weeks results in more on-treatment HBsAg decline than does 52 weeks of PEG-IFN alone. Continuation of ETV after PEG-IFN discontinuation may prevent

HBsAg rebound. Future randomized trials should evaluate the role of consolidation therapy.



P0670

PLASMA MICRORNA LEVELS ARE ASSOCIATED WITH THERAPY RESPONSE IN CHRONIC HEPATITIS B PATIENTS TREATED WITH PEGINTERFERON AND ADEFOVIR

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Background and Aims: MicroRNAs (miRNAs) are small, non-coding RNA molecules involved in various cellular processes of post-transcriptional regulation of gene expression. Circulating miRNAs have been linked to multiple diseases, including hepatitis B virus infection. The aim of this study was to quantify miRNAs in plasma of chronic hepatitis B (CHB) patients before the start of therapy and to determine miRNA signatures associated with response to antiviral therapy.

Methods: In an investigator-initiated study, 86 CHB patients completed 48 weeks of peginterferon alfa-2a and adefovir combination therapy, followed by a treatment-free follow-up of 2 years (week 144). We selected 12 HBeAg-positive and 12 HBeAg-negative patients as an identification cohort, both consisting of 6 non-responders and 6 combined responders (HBeAg negativity, HBV-DNA ≤2,000 IU/mL, and ALT normalization) of which 3 patients had HBsAg loss. A microRNA RT-qPCR assay (Exiqon) was used to determine the expression level of 179 different miRNAs in plasma. Data were pre-processed and analyzed with GenEx software from MultiD. MiRNAs with a >2-fold change between responders and non-responders ($p < 0.05$) were identified as potential candidates.

Results: In the identification cohort ($n = 24$), 21 miRNAs were differentially expressed between HBeAg-positive and -negative patients, of which 18 miRNAs were higher in HBeAg-positive patients. Patients with combined response had higher baseline levels of miR-142 compared to non-responders, whereas levels of miR-25-3p and miR-16-5p were lower. Patients with HBsAg loss had higher levels of miR-145-5p ($p < 0.01$) and miR-143-3p at baseline compared to non-responders, irrespective of HBeAg status. In HBeAg-positive patients, levels of 3 miRNAs were higher in patients with HBsAg loss compared to non-responders (miR-652-3p, miR-18b-5p and miR-221-3p), whereas miR-92a-3p level was lower. HBeAg-negative patients with HBsAg loss had a significant higher level of miR-143-3p and lower levels of 9 miRNAs (miR-30e-3p, miR-421, miR-146-5p and miR-26a-5p, all $p < 0.01$) at baseline compared to non-responders.

Conclusions: We identified several potential miRNA candidates that are associated with HBeAg status and HBsAg loss in CHB patients. Circulating miRNAs may be used as markers to predict treatment outcome in CHB patients receiving interferon-based therapy. The results obtained in these 24 patients are presently validated in a larger group of patients.

Viral hepatitis: Hepatitis C – a. Experimental (virology)

P0671

MECHANISMS OF HCV-INDUCED DIABETES THROUGH IMPAIRED INSULIN-DRIVEN GLUCONEOGENESIS SHUT-DOWN BY HCV PROTEINS AS A RESULT OF UNCOUPLING OF FoxO1/AKT SIGNALING

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Background and Aims: HCV infection is an independent risk factor for type 2 diabetes in humans. The underlying molecular mechanisms remain unknown. Our aims were to assess whether FL-N/35 transgenic mice expressing the full HCV-ORF develop diabetes, and to use this in vivo model to unravel the molecular mechanisms of HCV-induced diabetes.

Results: HCV transgenic mice displayed similar baseline glycemia, but significantly higher insulinemia and HOMA-IR than control littermates. Glucose tolerance tests resulted in significantly higher glycemia levels in transgenic mice, demonstrating that HCV protein expression is associated with glucose intolerance, as observed in HCV-infected patients. We measured insulin resistance and metabolic flux using hyperinsulinemic euglycemic clamps. We observed a significantly lower glucose infusion rate and higher endogenous glucose production (EGP), which were related to a drastic diminution of soleus striated muscle glucose uptake, witnessing insulin resistance in HCV mice at both muscular and hepatic levels. The insulin receptor pathway was studied in isolated HCV transgenic primary hepatocytes. We observed that the early steps of the insulin signaling pathway, from IRS1/2 to PDK1 phosphorylation, were constitutively impaired in the liver of HCV mice, potentially trough a SOCS3-dependent mechanism. Higher EGP in spite of higher fasting insulinemia in HCV mice was concomitant to lower levels of glucokinase and fructose-1,6-bisphosphatase and higher levels of phosphoenolpyruvate carboxykinase transcripts in their livers, a finding in keeping with enhanced gluconeogenesis. The lack of gluconeogenesis inhibition by insulin usually reflects abnormal AKT activation. Unexpectedly, AKT phosphorylation at serine 473 was increased in HCV mice compared to WT mice, even after insulin injection. However, phosphorylation of the forkhead transcription factor FoxO1 at serine 256, which triggers its nuclear exclusion, was lower in HCV mouse livers, demonstrating an uncoupling of the canonic AKT/FoxO1 pathway.

Conclusions: In conclusion, we showed that the sole expression of HCV proteins in the liver, in the absence of detectable inflammation, induces insulin resistance through an impairment of

insulin signaling leading to a pre-diabetic state in HCV transgenic mice, providing a valuable mechanistic explanation to HCV-induced diabetes in infected patients.

P0672

INTERACTION BETWEEN HEPATIC MEMBRANE TYPE-1 MATRIX METALLOPROTEINASE AND ACIREDUCTONE DIOXYGENASE 1 REGULATES HEPATITIS C VIRUS INFECTION

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Background and Aims: Membrane type-1 matrix metalloproteinase (MT1-MMP or MMP14) binds to and regulates the function of tetraspanin-enriched microdomains, which comprised members of the tetraspapnin family, including CD81. MMP14 also physically interacts with two other proteins, claudin-1 and acireductone dioxygenase 1 (ADI1), which are associated with hepatitis C virus (HCV) cell entry. To gain insight into the regulatory role of MMP14 in HCV infection, we examined the liver expression levels of MMP14, ADI1, and claudin-1 in association with hepatic HCV-RNA amounts.
Methods: 104 liver biopsies obtained from chronic hepatitis C patients and 84 liver tissues derived from non-cancerous parts of surgically removed HCV-related hepatocellular carcinomas (HCC) were included. Protein expression levels were assessed by IHC and/or western blot. Physical association between MMP14 and ADI1 or claudin-1 was assessed by co-immunoprecitation.
Results: Neither the tissue ADI1, claudin-1, or the tissue MMP14 levels correlated with hepatic HCV-RNA amounts. However, in chronic hepatitis C patients, the presence of cytoplasmic localization of ADI1 in liver biopsies was associated with a higher serum HCV RNA level (P = 0.009). In HCC tissues, 45 (53.6%) samples tested positive for MMP14 – ADI1 interaction harbored a lower HCV-RNA amounts (2.22±1.61 vs. 3.10±1.40 Log IU/gram, P = 0.009). The HCV-RNA amounts in this group of patients were positively associated with the ADI1 levels in the MMP14 – ADI1 co-immunoprecipitates (P = 0.030). On the other hand, in 33 (39.3%) samples tested positive for MMP14 – claudin-1 interaction, the hepatic HCV-RNA amounts were positively correlated with the claudin-1 levels in the MMP14 – claudin-1 co-immunoprecipitates (P = 0.033). Over-expression of MMP14 in Huh7.5 cells suppressed cell entry of HCV pseudoparticles as well as HCVcc infection. The suppression effect could be reversed by co-expression of ADI1 in a dose-dependent manner.
Conclusions: MMP14 – ADI1 interaction was associated with down-regulation of HCV infection. The negative regulatory effect could be reversed by over-expression of ADI1. Our findings supported a role of tetraspanin-enriched microdomains in HCV cell entry.

P0673

PATHOPHYSIOLOGY OF HCV-RELATED HEPATOCELLULAR CARCINOMA: HCV PROTEIN EXPRESSION INDUCES THE ACTIVATION OF AKT1 IN HEPATOCYTES THROUGH AN mTORC2 DEPENDENT PATHWAY

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Background and Aims: The Pi3K-AKT pathway is a critical intracellular node regulating cell survival and proliferation. Activation of the AKT pathway has been reported in many cancers, including hepatocellular carcinoma (HCC). We previously showed that c-MYC is overexpressed through an AKT-dependent mechanism in HCV-infected patients and transgenic mice expressing the HCV proteins (*Higgs et al., Oncogene 2013*). The present study aimed at unraveling the molecular mechanisms of AKT activation during HCV infection.
Methods: AKT activation pathway was studied in human and mouse liver samples by mean of immunoprecipitation and western blotting.
Results: We observed a significant hyperphosphorylation of AKT-ser473 in non-tumoral hepatic tissues from infected patients with HCC as compared to HBV-infected or non-infected alcoholic patients. Hyperphosphorylation of AKT-ser473 was also observed in 3 months-old HCV transgenic mouse livers as compared to wild-type mice, which persisted after EGF treatment. AKT1, but not AKT2, was the activated form. We observed an increase in the phosphorylation of mTOR-ser2448 within the mTORC2 complex and a concomitant increased kinase activity of this complex, associated with reduced phosphorylation of p70S6K-thr389 and PDK1-ser241.
Conclusions: Our results suggest that HCV protein expression modulates the negative feedback loop that controls AKT phosphorylation, thus leading to its hyperactivation, potentially playing a role in hepatic carcinogenesis. Because numerous molecules targeting the PI3K/AKT pathway have been used in other cancers than HCC, our results suggest such approaches could be valuable in the prevention or treatment of HCV-associated HCC.

P0674

CLINICALLY APPROVED T-TYPE CALCIUM CHANNEL INHIBITORS PREVENT HEPATITIS C VIRUS (HCV) MEMBRANE FUSION IN A GENOTYPE-DEPENDENT MANNER

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Background and Aims: Novel directly acting antivirals have greatly improved treatment options for chronically infected HCV patients. However, due to resistance development these drugs cannot be used in monotherapy necessitating combination therapies. While several highly effective drug combinations are licensed, these regiments are costly thus limiting therapy access. The aim of this study was to identify alternative FDA-approved drugs with antiviral activity and novel mode of action against HCV.
Methods: We screened a library of licensed drugs for antiviral activity against HCV using an HCV whole life cycle assay. Antiviral activity of selected compounds was validated using wild type HCV, primary human hepatocytes and genetically humanized mice. The mode of action of compounds was assessed using cell entry, membrane fusion, RNA replication and virus assembly assays. Breadth of HCV-specific antiviral activity and viral target genes were determined using chimeric HCV constructs from genotypes 1 through 7. Drug resistance was explored by extensive cell culture passage, sequencing, reverse genetics and functional assays.
Results: Flunarizine, a T-type calcium channel inhibitor used to treat migraine specifically inhibited HCV cell entry (IC50 of 388.2 nM) in Huh-7 cells and in primary human hepatocytes. It also reduced HCV infection in a genetic mouse model for HCV cell entry. Interestingly, genotype 2a (GT2a) cell culture-derived particles (HCVcc), but not non-GT2a viruses were susceptible to

flunarizine. Shuffling structural proteins between GT1a, GT2b and GT2a chimeric viruses revealed that GT2a-derived E1-E2 genes confer susceptibility to flunarizine. Resistance mutations map to the viral envelope proteins, thus indicating that flunarizine targets glycoprotein function(s). Pretreatment of target cells, but not of virus particles inhibited HCV entry and time of addition experiments indicated that flunarizine acts at a late stage of entry. Since flunarizine interfered with HCV infection through low-pH-triggered fusion at the plasma membrane, we conclude it inhibits HCV independent of endocytosis. Finally, trans-complemented HCV particles (HCV-TCP) carrying glycoproteins from GT2a patients were inhibited by flunarizine and flunarizine-resistant viruses were more prone to neutralization by patient-derived antibodies.
Conclusions: Flunarizine may be an alternative treatment option for GT2a- and possibly other GT2-subtype infected individuals.

P0675
AN EX VIVO MODEL OF HUMAN LIVER SLICES CULTURE FOR EVALUATING LIVER FIBROGENESIS

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Background and Aims: We extended our *ex-vivo* model of human liver slices culture infected with HCV (Hepatology 2012) from 10 to 21 days and evaluated the markers of fibrosis in HCV-infected liver slices as compared to uninfected liver slices (CRTL) in presence of Ethanol or not.
Methods: Non-infected liver slices, obtained from human liver resection and cut in 350 μm-thick slices (2.7×10^6cells per slice), were cultivated for up to 21 days: either in presence or not of ethanol (EtOH) (1mM, 5mM, 25mM), or infected with HCVcc supernatant [Con1/C3 (genotype1b)] (MOI = 0.1) in presence or not of ethanol (1mM, 5mM, 25mM). Expression of liver phenotypic markers and fibrosis markers [Tumor growth factor beta (TGFβ), Heat shock protein 47 (Hsp47), Alpha smooth muscle actin (αSma) and Procollagen1 A1 (Procl1A1)] were checked by RT-qPCR. The amount of Hyaluronic acid and TGFβ were determined by ELISA assays.
Results: Hepatocyte-specific gene expression is maintained in human non infected liver slices culture for 21 days. In uninfected liver slices treated with EtOH, the gene expression of TGFβ, Hsp47, αSma and Procl1A1 increased overtime, in a dose-dependent manner up to 3, 3, 4, 4 times, respectively, at day 21, at 25mM EtOH, compared to uninfected non treated control liver slices. Human infected liver slices replicated, expressed efficiently HCVcc and produced high titers of progeny virions (HCVpc) by day 21. In HCV infected liver slices, the gene expression of TGFβ, Hsp47, αSma and Procl1A1 were increasing overtime and exhibited at day 21, a significant 3, 3.5, 3.6, 2.6 fold increase, respectively as compared to uninfected liver slices. At day 10, the amount of hyaluronic acid (HA) reached 799 μg/L, more than a 7-fold increase compared to uninfected slices. At day 21, the expression of the TGFβ protein, intracellular and in the culture supernatant, were significantly 4 and 2 times increased (up to 152 pg/mg tissue and 1100 pg/mg tissue, respectively) as compared to uninfected liver slices. In HCV infected liver slices treated with EtOH, the gene expression of TGFβ, Hsp47, αSma and Procl1A1 increased significantly in a dose-dependent manner, up to day 21, around 5, 6, 7, 5 times, respectively, at 25mM EtOH, compared to controls.
Conclusions: This *ex vivo* model, supporting hepatocyte-specific gene expression for 21 days, provides a powerful tool for studying the onset of fibrosis and for evaluating the potency of new antifibrotic therapies in the native tissue.

P0676
CLEARANCE OF PERSISTENT HEPATITIS C VIRUS INFECTION USING A MONOCLONAL ANTIBODY SPECIFIC FOR TIGHT JUNCTION PROTEIN CLAUDIN-1

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Background and Aims: Hepatitis C virus (HCV) infection is a leading cause of liver cirrhosis and cancer. Although direct-acting acting antivirals have revolutionized treatment, several challenges remain: these include treatment of certain genotypes, advanced liver disease, resistance and liver graft infection. Tight junction (TJ) proteins claudin-1 and occludin mediate cell entry of HCV. However, the role of TJ proteins as therapeutic target is unknown.
Methods: Using a human liver-chimeric mouse model combined with advanced *in situ* imaging and mechanistic studies we investigated the role of TJ protein claudin-1 as a therapeutic target for HCV infection.
Results: Here we report that a monoclonal antibody specific for TJ protein claudin-1 eliminates chronic HCV infection with undetectable resistance and toxicity in a human liver chimeric mouse model. In contrast to DAAs the claudin-1 specific antibody can cure infection in monotherapy. This antibody inhibits HCV entry, cell-cell transmission and virus-induced signaling events. Importantly, antibody treatment reduces the frequency of HCV-infected hepatocytes *in vivo*, highlighting the need for *de-novo* infection via host entry factors to maintain chronic infection.
Conclusions: We demonstrate that an antibody targeting a virus receptor can cure chronic HCV infection and uncover TJ proteins as targets for antiviral therapy. This host-targeting strategy provides a simple approach for prevention of liver graft infection and opens a novel perspective for treatment of drug resistance.

P0677
CIVACIR HEPATITIS C IMMUNE GLOBULIN (HCIG) POTENTLY NEUTRALIZES INFECTION OF HEPATITIS C VIRUS TRANSPLANT ESCAPE VARIANTS

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Background and Aims: Hepatitis C virus (HCV) induced end-stage liver disease is the major indication for liver transplantation in most countries. However, re-infection of the liver graft is universal. The safety and efficacy of DAAs for prevention of liver graft infection remains to be determined. Biotest Pharmaceutical's Civacir, a human hepatitis C antibody enriched immune globulin product (HCIG), has been shown to efficiently prevent liver graft infection in a phase III RCT (Terrault et al. AASLD 2014). Using well characterized patient-derived HCV transplant escape variants (Fofana et al. Gastroenterology 2012; Felmlee/Fauvelle et al. EASL 2014) we aimed to study the molecular mechanism of action of HCIG/Civacir.
Methods: Inhibition of Civacir/HCIG-mediated HCV infection was studied using 25 viral variants isolated from patients before and after liver transplantation and state-of-art HCV cell culture models. HCV pseudoparticles (HCVpp) and cell-culture-derived HCV (HCVcc) expressed patient-derived viral envelope glycoproteins from transplant escape variants.
Results: Civacir potently, broadly and dose-dependently neutralized all the patient variants in HCVpp assays including variants displaying a high entry phenotype and resistant to host neutralizing antibodies. The IC50 values (0.0016–1.50 mg/ml) for inhibition were independent of the phenoype of the viral variant indicating that virus neutralization by Civacir/HCIG is not affected by viral escape. Furthermore, at clinically relevant antibody concentrations Civacir potently neutralized HCVcc variants bearing envelopes efficiently escaping patient-derived and monoclonal anti-E2 antibodies.
Conclusions: Patient-derived HCV escape variants resistant to autologous antibodies are potently neutralized by Civacir in state-of-the-art HCV cell culture models. The potent activity of Civacir is likely because of synergy between anti-HCV antibodies derived from different patient donors. Collectively, these results uncover the mechanism of action of HCIG/Civacir and explain its clinical efficacy for prevention of HCV liver graft infection.

P0678
IL-8 PRODUCING CD4+ REGULATORY T CELLS STIMULATE ANGIOGENESIS IN CHRONIC HEPATITIS C

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Background and Aims: In chronic hepatitis C regulatory $CD4^+$ T cells (Tregs) inhibit anti-viral T cell response via soluble IL-10 and IL-35 (Langhans et al., Clin Science 2010). In addition, we demonstrated that $CD4^+$ Tregs enhance fibrosis by modulating the interaction of NK cells and hepatic stellate cells (HSC) and stimulating fibrogenesis in HSC by secreting IL-8 (Langhans et al., J Hepatol 2014 in press). Here, we analyzed in how far intrahepatic $CD4^+$ Tregs can stimulated angiogenesis.
Methods: Biopsies and explant livers from patients with chronic hepatitis C (cHCV), chronic hepatitis B (cHBV) and alcoholic liver disease (EtOH) were studied by multiple colour immunofluorescence histology on cryostat sections. Numbers of IL-8^+Foxp3^+CD127^{low}CD25^+CD4^+ Tregs were determined quantitatively using flow cytometry in freshly isolated liver infiltrating lymphocytes obtained from unfixed parts of the liver samples.
Results: Our quantitative *ex vivo* analysis revealed that HCV-specific Tregs produce marked amounts of IL-8 (MW±SEM: 650±133 pg/ml). Despite equivalent numbers of $CD4^+$ Tregs, IL-8 producing Tregs were exclusively found in the liver of patients with cHCV, but not cHBV or EtOH. *In situ* we found a close relationship between Tregs and intrahepatic blood vessels indicating that activated Tregs are focally associated with $C31^+$ endothelial cells in HCV-associated cirrhosis. Of note, Tregs attached to the vessels from outside and not the lumen. In the liver tissue of HCV-patients overall number of $CD4^+$ Tregs were significantly enhanced with advanced fibrosis and correlated with IL-8 producing Tregs ($R = 0.7501$, $p < 0.001$). Further *in vitro* experiments demonstrated that HCV-specific Tregs produce additional potent angiogenic factors such as MMP8, MMP9, TIMP-1 and activin-A and serpin-E1.
Conclusions: Our data propose that intrahepatic $CD4^+$ Tregs are associated with blood vessels in the liver of patients with chronic hepatitis C and thus have the potential to stimulate angiogenesis by producing IL-8.

P0679
DERISKING THE POTENTIAL FOR MITOCHONDRIAL TOXICITY OF NUCLEOSIDE ANALOGS

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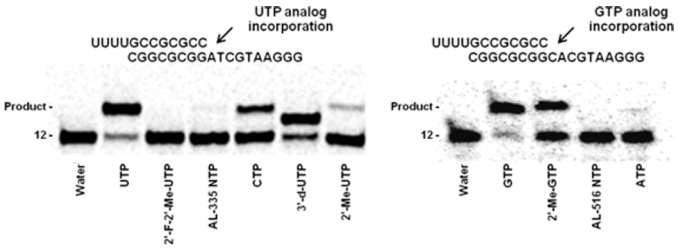
Background and Aims: Nucleoside analogs play an important role in the treatment of multiple viral diseases. Nucleoside analogs that fail clinically do so for multiple reasons, but primarily for lack of selectivity versus human polymerases resulting in mitochondrial toxicity. In general, nucleoside toxicity appears compound specific and does not appear to be associated with any particular base, sugar or phosphate modification. We have established a screening paradigm designed to assess the potential for mitochondrial toxicity of ribonucleoside analogs. We exemplified the strategy using nucleoside analogs previously advanced for the treatment of chronic hepatitis C (CHC), and applied the strategy to novel nucleoside analogs currently being advanced for the treatment of CHC.
Methods: The triphosphate derivative (NTP) of each nucleoside analog was prepared and evaluated for the ability to be incorporated by human mitochondrial RNA polymerase (HMRP). Nucleoside analogs were tested in HepG2 cells for 6–8 days for inhibition of the synthesis of the mitochondrial-DNA encoded protein, cytochrome c oxidase I (COX-I), relative to the nuclear DNA encoded mitochondrial protein, succinate dehydrogenase A subunit (SDH-A). Nucleoside analogs were also assessed for general cytotoxicity in 8-day assays across a panel of cell lines.
Results: The NTP's of 4′-azidocytidine, INX-08189, PSI-7851, AL-335 and AL-516 all showed potent inhibition of the HCV NS5B polymerase. 4′-azidocytidine and 2′-Me-guanosine NTP's were shown to be efficient substrates for incorporation by human mitochondrial RNA polymerase, the NTP's of 2′-Me-2′-F-uridine, AL-335 and AL-516 were not substrates for HMRP (Figure). 4′-azidocytidine and 2′-Me-guanosine prodrugs were inhibitors of human mitochondrial protein synthesis. 2′-Me-2′-F-uridine prodrug PSI-7851, AL-335 and AL-516 did not inhibit human mitochondrial protein synthesis. For most cell lines, only 2′-Me-guanosine prodrugs showed cytotoxicity.

Conclusions: Nucleoside analogs that metabolize to NTP's that are strongly incorporated by human mitochondrial RNA polymerase demonstrate potent inhibition of mitochondrial protein synthesis and have demonstrated an undesirable clinical safety profile. AL-335 and AL-516 are not substrates for the human mitochondrial RNA polymerase and do not inhibit mitochondrial protein synthesis. These compounds are being advanced in clinical and preclinical studies for the treatment of patients infected with CHC.



P0680

HEPATITIS C VIRUS ACTIVATES AUTO- AND PARACRINE CIRCUITS TO SWAY SURFACE EXPRESSION OF ErbB FAMILY MEMBERS

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Background and Aims: Recently the EGF receptor and EGFR dependent signalling has been identified as a co-factor for HCV entry and replication. In how far HCV also interferes with expression and signal-transduction of the other three members of the EGF receptor family is unknown. The present study analyses the influence of HCV on the surface expression of the other members of the EGF receptor family and their consequences.
Methods: Huh7 cells either infected with the HCV cc strain JC1 or harbouring the HCV1 subgenomic replicon were used and analysed employing targeted gene knockdown by siRNA, rtPCR and Immunoblot-analysis.
Results: The data presented indicate that HCV, Sp1-dependently enhances the expression of Nrg1 and that this is required to down-regulate ErbB3 expression via an Nrg1-mediated autocrine circuit. This HCV-mediated reduction of ErbB3 expression in turn mediates enhanced surface expression of EGFR and ErbB2, an effect that can be mimicked by siRNA-mediated knockdown of ErbB3 expression. This seems to favour viral replication as suggested from the fact that pre-treatment with NRG1 as well as knockdown of ErbB3 expression prior to infection with the HCVcc strain JC1 results in an increased abundance of viral transcripts.
Conclusions: These data delineate a novel mechanism enabling HCV to modify the composition of the ErbB family members on the surface of its host cell by a paracrine NRG1-driven circuit. The resulting up-regulation of EGFR and ErbB2 may be in advantage for viral life cycle.

P0681

CATALASE ACTIVITY INHIBITION BY HEPATITIS C VIRUS (HCV) IN HCV-INDUCED OXIDATIVE STRESS, A TRIGGER OF HEPATIC CARCINOGENESIS

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Background and Aims: Hepatitis C virus (HCV) infection is a major risk factor for the onset and progression of hepatocellular carcinoma (HCC). Chronic HCV infection induces the accumulation of reactive oxygen species (ROS) in infected livers leading to oxidative stress. Little is known about alterations of intracellular antioxidant defense pathways involved in HCV-related oxidative stress. Cellular catalase is an enzyme that detoxifies hydrogen peroxide (H_2O_2), a ROS known to control cell proliferation, thus playing a major role in the cellular antioxidant defense mechanism.
The aim of this study was to characterize the expression and activity of cellular catalase, using different *in vitro* and *in vivo* models of HCV replication or HCV protein expression.
Results: Cirrhotic liver samples from HCV-infected patients exhibited a significant decrease of their intracellular catalase activity when compared to alcoholic and HBV-infected cirrhotic liver samples. However, the intrahepatic catalase protein levels were not different between these three groups of patients. In order to explore the impact of HCV protein expression *in vivo* in the absence of local inflammation and immune response, the transgenic (tg) mouse model expressing the full-length HCV ORF (FL-N/35 mice) was used. Tg mouse livers display high levels of oxidative stress. We observed a significantly higher oxidative nuclear DNA damage, together with an increase in the amount of advanced oxidation protein products, in tg livers compared to their wild type (wt) littermates. Like in human samples, the level of H_2O_2 detoxification in tg mice livers was weak. Primary hepatocytes isolated from tg mice displayed a dramatic, up to 80%, reduction of catalase activity when compared to wt mice. Remarkably, catalase expression (mRNA and protein levels) was similar in tg and wt liver samples and isolated primary hepatocytes. The JFH1-based cell-culture system was used to assess catalase expression and activity in a fully replicating system. Like in the other models, a dramatic increase in the intracellular oxidative stress level was observed in HCV-infected cells, concomitant to a significant decrease of catalase activity without alteration of catalase expression.
Conclusions: HCV infection is responsible for a default in intracellular catalase activity, as a result of HCV protein expression in the absence of local inflammation. This mechanism could, at least in part, contribute to the HCV-induced intracellular oxidative stress, a major trigger of HCC.

P0682

PRECLINICAL CHARACTERIZATION OF AL-335, A POTENT URIDINE BASED NUCLEOSIDE POLYMERASE INHIBITOR FOR THE TREATMENT OF CHRONIC HEPATITIS C

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Background and Aims: Nucleotide analog HCV polymerase inhibitors have demonstrated a high barrier to resistance and are a key component of some interferon-free combination regimens for the treatment of chronic hepatitis C (CHC). We identified AL-335 as part of our efforts to advance potential medicines for the treatment of CHC. AL-335 is a novel, potent uridine based nucleotide analog that demonstrates a desirable preclinical profile.
Methods: The antiviral activity and selectivity of AL-335 were evaluated in vitro using the HCV replicon system encoding NS5B sequences from multiple genotypes and resistant variants.
AL-335 was also studied in replicon in combination with other compounds. The nucleoside triphosphate (AL-335 NTP) was tested for potency versus the recombinant HCV polymerase NS5B, and for selectivity against human DNA and RNA polymerases. NTP incorporation assays were conducted using HCV NS5B polymerase and the AL-335 NTP to assess the mechanism of action. Intracellular formation and half-life of the AL-335 NTP in primary human hepatocytes was assessed. AL-335 was studied in vivo following

oral dosing for the ability to form the active AL-335 NTP in dog liver.
Results: In the stable genotype (GT) 1b replicon assay, AL-335 exhibited potent antiviral activity with an EC_{50} of 75 nM. In transient chimeric GT-1b replicons with NS5B regions derived from GT 1–4, AL-335 demonstrated pan-genotypic activity with EC_{50} values between 40–60 nM. AL-335 retained activity vs. replicon mutants resistant to non-nucleoside polymerase, NS3/4A protease and NS5A inhibitors. AL-335 was additive or synergistic in all combinations studies. The AL-335 NTP was a potent inhibitor of the HCV NS5B polymerase with an IC_{50} of 120 nM and a K_i of 24 nM, and acted as a chain-terminator of RNA synthesis. The AL-335 NTP was not a substrate for human mitochondrial RNA polymerase and demonstrated no inhibition (IC_{50} >100 μM) of human DNA or RNA polymerases. In vitro, the AL-335 NTP was rapidly formed and demonstrated a long intracellular half-life of >24 hrs in primary human hepatocytes. Following oral administration to dog at 5 mg/kg parent nucleoside equivalent, the AL-335 NTP was formed at high and sustained levels in liver (AL-335 NTP levels at 24 hrs: 2.1 μM).
Conclusions: AL-335 is a potent uridine based nucleotide analog that demonstrates a desirable preclinical profile. The compound is currently advancing in preclinical studies as a potential treatment for CHC.

P0683
HEPATITIS C VIRUS NS3-4A PROTEASE TARGETS THE HOST FACTOR BNIP1 AT A NON-CANONICAL CLEAVAGE SITE
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Background and Aims: The hepatitis C virus (HCV) NS3-4A protease plays a central role not only in the viral life cycle but also in the persistence and pathogenesis of HCV. It cleaves selected host factors, thereby modulating cellular signaling pathways. Here, we describe BNIP1 as a novel cellular substrate of the HCV NS3-4A protease.
Methods: A large-scale quantitative proteomics screen was performed to identify novel cellular substrates of the HCV NS3-4A protease. Candidates were validated in a wheat germ-based cell-free and various cellular expression systems as well as in Huh-7 human hepatocellular carcinoma cells harboring subgenomic HCV replicons or infected with cell culture-derived HCV (HCVcc).
Results: BNIP1 (BCL2/adenovirus E1B 19 kDa interacting protein 1), a BH3-only transmembrane protein, was identified as a novel cellular substrate of the NS3-4A protease. BNIP1 is localized on the outer mitochondrial membrane and has been involved in apoptosis, autophagy, and other cellular processes of potential relevance to HCV infection. Coexpression of N-terminally FLAG-tagged BNIP1 with active but not with catalytically inactive NS3-4A protease resulted in the cleavage of BNIP1 into 3 defined fragments both in a cell-free expression system and in transfected cells. Cleavage was abrogated by the protease inhibitor telaprevir, further demonstrating that it depends on the presence of active NS3-4A protease. Cleavage of endogenous BNIP1 was also observed in cells harboring subgenomic HCV replicons and in cells infected with HCVcc. Mutagenesis of Cys and Thr residues within BNIP1 did not abrogate cleavage by the NS3-4A protease, suggesting the presence of a non-canonical cleavage site. Indeed, mass spectrometry and validation by site-directed mutagenesis identified BNIP1 Ser 166 as residue targeted by the NS3-4A protease.
Conclusions: Quantitative proteomics identified BNIP1 as a novel host factor targeted by the HCV NS3-4A protease at a non-canonical cleavage site. Ongoing experiments explore the role of BNIP1 and its cleavage in the life cycle and pathogenesis of HCV.

P0684
MODULATION OF HEPATITIS C VIRUS INFECTION THROUGH KHSRP-DEPENDENT REGULATION OF MIRNA-122 MATURATION AND INTRACELLULAR RNA DEGRADATION
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Background and Aims: During infection, the hepatitis C virus (HCV) genome is exposed to degradation by the cellular RNA-decay machinery. Indeed, HCV RNA is a target for cellular RNAses due to the lack of a 5′ methylated cap and of a 3′ polyA tail and to the presence of a destabilizing RNA element (the 3′ polyU region). miR-122 has been shown to play a role in protecting the HCV genome from 5′-end degradation. The goal of this work was to unravel the role of KHSRP, an AU-Rich Element mRNA-binding protein capable to recruit 5′- and 3′-RNAses such as XRN1 and DIS3 respectively, and participating in miRNA maturation, during HCV infection.
Methods: Transient gene knockdown was performed by means of siRNA transfection. Translation efficiency was measured by means of luciferase non-replicative constructs containing viral UTRs. RNA stability was evaluated by Northern-Blot analysis. Viral replication was measured using a modified JFH1 strain expressing Gaussia luciferase. Gene and miRNA expression was quantified by qRT-PCR. The post-translational modifications and subcellular localization of KHSRP were studied by means of *in situ* Proximity Ligation Assay (PLA).
Results: XRN1 and DIS3 silencing increased JFH1 replication in Huh7 cells and the stability of nonreplicative viral RNA, whereas KHSRP silencing inhibited both viral replication and degradation of nonreplicative HCV RNA. Because KHSRP is involved in miRNA maturation, we studied miR-122 expression in the context of KHSRP silencing and expression. KHSRP expression dramatically increased miR-122 maturation. Using nonreplicative JFH1 variants and the con1 SubGenomic Replicon (SGR), we showed that the expression of structural HCV proteins decreased the amount of KHSRP mRNA. In HCV-SGR cell line, the AKT-dependant phosphorylation of KHSRP at position Ser193 led to the nuclear relocalization of phospho-KHSRP (Figure 1) concomitantly to the maturation of nuclear pri-miR-122. PLA showed the co-localization of KHSRP, the HCV NS5A protein and HCV RNA in the cytosol.

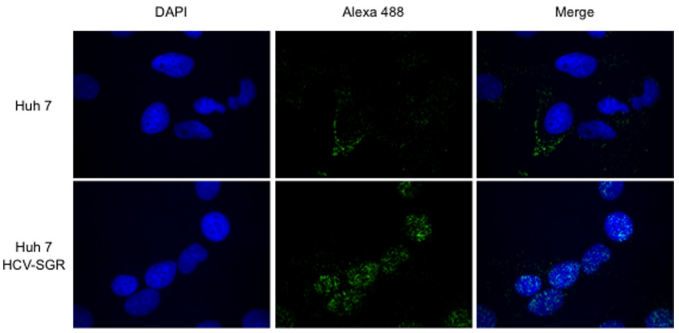

Figure 1. Nuclear relocalization of Phospho Ser^{193}-KHSRP in Huh7 cells containing the HCV Con1 Sub Genomic Replicon (HCV-SGR) by *in situ* Proximity Ligation Assay.

Conclusions: Our results show that HCV down-regulates KHSRP gene expression in hepatocytes, thus protecting HCV RNA from intracellular degradation by XRN1 and DIS3 RNAses. HCV infection also leads to the phosphorylation and relocalization of phospho-KHSRP to the nucleus, where it induces miR-122 maturation,

thereby increasing HCV RNA stability and favoring replication. This thus far unknown regulatory mechanism at the same time promotes viral replication through miR-122 maturation and limits viral RNA degradation.

P0685

THE ROLE OF CAVEOLIN-1 IN THE PHENOTYPE OF HUMAN T LYMPHOCYTES AFTER UPTAKE OF HCV NON-ENVELOPED PARTICLES

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Background and Aims: The existence of Hepatitis C (HCV) non-enveloped particles (HCVne) circulate in the serum of HCV infected patients has been correlated with viral persistence. Previous study from our group demonstrated that recombinant HCVne particles can efficiently enter PBMCs with concomitant secretion of cytokines leading them to partial T cell exhaustion and rise of Tregs. Caveolin-1 is a protein that plays important role in the signaling events of the cells. Caveolin-1 role in T lymphocytes is still under investigation. The aim of this study was to investigate the role of caveolin-1 in the above mechanisms/response.
Methods: PBMCs were isolated from the peripheral blood of healthy donors. Using the baculovirus expression system we have generated recombinant HCVne in the absence of other viral proteins. PBMCs were incubated either with HCV non-enveloped particles (HCVne) or the control fraction for 12 h, 24 h, 48 h and 66 h. Expression of caveolin-1, CD107a (cytotoxicity) and T regulatory cells (CD4+CD127-CD25+) were estimated by flow cytometry in all samples.
Results: Caveolin-1 expression was increased in PBMCs after 48 h incubation with HCVne compared to the control fraction group (12.59% vs 10.7%). There was no change in the caveolin-1 expression at all time points in the control group. The percentage of CD4+ T cells expressing caveolin-1 (CD4+cav1+) was significantly increased at 48 h compared to the respective percentage at 24 h in the HCVne group (2.53% vs 1.65%, $p = 0.025$). After 66 h incubation with HCVne, %CD4+cav1+ were positively correlated with %Tregs ($r = 0.957$, $p = 0.040$) and %CD4+CD25+ T cells ($r = 0.941$, $p = 0.017$) and negatively correlated with %CD107 expression after 48 h ($r = -0.893$, $p = 0.042$). Moreover, the percentage of %CD8+cav1+ after 12 h, 48 h and 66 h were negatively correlated with %CD107a expression ($r = -0.996$, $p = 0.001$, $r = -0.884$, $p = 0.046$ and $r = -0.953$, $p = 0.012$, respectively).
Conclusions: Caveolin-1 seems to play a crucial role in the establishment of the partial exhausted phenotype of T lymphocytes after HCVne incubation.

P0686

RECIPROCAL ANTAGONISM BETWEEN THE UNCOORDINATED PHENOTYPE-5A (UNC5A) DEPENDENCE RECEPTOR AND THE HEPATITIS C VIRUS

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Background and Aims: Hepatitis C virus (HCV) infection is a leading cause of hepatocellular carcinoma (HCC), mainly through cirrhosis induction. Cirrhosis may be detrimental for SVR rates upon usage of recently developed direct acting antivirals (DAAs), spurring research for a deeper understanding of HCV infection in cirrhosis. This study investigated the interplay between HCV and UNCA, a dependence receptor that is inactivated in cancer.
Methods: The impact of HCV on UNC5A expression was evaluated on liver resections from three cohorts of chronically HCV-infected, HBV-infected and uninfected patients ($n = 503$) and on in vitro JFH1, H77 and N strain-infected Huh7.5 cells and primary human hepatocytes (PHH). Conversely, the impact of UNC5A expression on HCV replication parameters was evaluated in UNC5A RNAi and forced expression approaches.
Results: UNC5A expression is significantly decreased in HCV(+) but not HBV(+), liver biopsies as compared to uninfected samples. UNC5A is downregulated in F2 (3-fold; $p = 0.003$) and more dramatically in F4/cirrhosis (44-fold; $p < 0.0001$) histological stages in HCV(+) hepatic lesions compared to histologically-matched HCV(−) tissues. This down-regulation is not reversible upon SVR. UNC5A expression is significantly decreased by HCV genotypes *in vitro*, through decreased mRNA transcription and translation in vitro and in patients. The NS3/4A protease is involved in UNC5A downregulation. Knockdown and forced-expression experiments identified UNC5A as capable of HCV restriction through impeded morphogenesis, release, and specific infectivity of HCV virions, in a DAPK-dependent and cell-death independent manner. Finally, UNC5A was found strongly downregulated in HCC and UNC5A modulation conditioned cell survival of apoptosis-sensitized Huh7.5 and PHH cells, suggesting its implication in hepatocytic turn-over.
Conclusions: UNC5A-depleted hepatocytes by HCV may acquire selective advantage for ongoing viral replication and at the same time resistance to cell death, especially at the cirrhosis stage, a condition strongly exposed to HCC development.

P0687

REGULATION OF HEPCIDIN (HAMP) AS DRIVING FORCE FOR MACROPHAGE-MEDIATED HEPATITIS C (HCV) PERSISTENCY

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Background and Aims: What favours viral persistence over clearance during the acute phase of HCV infection remains unclear, but deregulated cellular and humoral immune responses are involved. Chronic HCV infection is associated with hepatic iron

overload and hyperferraemia that also correlate to poor antiviral responses. Hepcidin controls iron homeostasis and is increased by inflammation and iron excess. Chronic HCV patients possess low hepcidin levels, but HAMP knock-down has been linked to altered HCV replication.
We aimed to: (a) assess the interplay between HCV and hepcidin during acute and chronic infection, (b) investigate whether any putative hepcidin modulation alters iron homeostasis in macrophages, key players both in iron regulation and inflammation initiation, thereby favouring viral persistence.
Methods: Indirect co-cultures of liver (Huh7.5)–macrophage (THP-1) cells and patients' sera were employed to investigate the effect of HCV infection on hepcidin *in vitro* and *in vivo*. Hepcidin-expressing hepatoma cells transfected with HCV replicons were used to assess HCV translation and replication.
Results: HAMP expression was up-regulated early in HCV infection, dropping at later times. Ferritin and ferroportin levels changed accordingly. Hepcidin overexpression enhanced both viral translation and replication. In HCV-infected co-cultures, increased HAMP expression was observed. Ferritin was diminished in Huh7.5 cells with time, while a concurrent increase was observed in THP-1. This change in iron content was accompanied by 2–3 HCV replication rounds in Huh7.5 cells and one round in THP-1. Iron-loading of THP-1 reversed the observed ferritin "flow" and resulted in enhancement of hepatic HCV replication. Moreover, both macrophage populations managed to infect naïve Huh7.5 cells, but infection occurred faster in the presence of iron. Hepcidin changes were independent of IL-6 expression. Finally, hepcidin serum levels were significantly increased in acutely infected patients, but correlated with viral load levels in chronic patients.
Conclusions: HCV differentially modulates HAMP expression in both acute and persistent chronic infection *in vivo* and *in vitro*, thereby supporting its own life cycle. Hepcidin regulation may promote viral persistence, since iron level changes in both macrophages and hepatocytes render the former "viral reservoirs", able to support low levels of replication of infectious transmittable virus.
P. Foka and A. Dimitriadis contributed equally.

P0688

PRECLINICAL CHARACTERISATION OF MIV-802, A NOVEL URIDINE NUCLEOTIDE HCV NS5B POLYMERASE INHIBITOR, FOR TREATMENT OF HEPATITIS C VIRUS INFECTION

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Background and Aims: HCV NS5B polymerase nucleotide inhibitors have pan-genotype activity and high barriers to resistance and are therefore considered to be central to future DAA combination therapies for HCV. MIV-802 is the protide of a novel uridine analogue that is being developed for HCV therapy.
Methods: Antiviral activity was evaluated using chimeric HCV replicons expressing NS5B sequences from HCV genotypes 1–6, including resistance mutations conferring to resistance to nucleotides and other classes of HCV antivirals, and using clinical isolates (genotypes 1–4). The cellular and mitochondrial toxicity and the genotoxicity of MIV-802 and its parent nucleoside were characterized using a panel of cell lines and human primary cells. The uridine nucleoside triphosphate (MIV-802-UTP) was tested against recombinant HCV NS5B polymerase and against human RNA and DNA polymerases. MIV-802-UTP levels were determined in primary human hepatocytes *in vitro* and in dog liver after oral dosing.
Results: MIV-802-UTP is a potent and selective chain-terminating inhibitor of HCV NS5B polymerase with a Ki of 0.71 μM, but has IC_{50} >200μM against human DNA polymerases α, β and γ. MIV-802 displays pan-genotypic potency in replicon assays with an EC_{50} range of 22–74 nM, and against clinical isolates with an EC_{50} range of 17–108 nM. MIV-802 retains potency against mutant replicon variants resistant to the other HCV drug classes. The CC_{50} values for MIV-802 and its parent nucleoside are >50 μM when tested against a broad panel of human cell lines including fresh human hepatocytes, bone marrow progenitor cells and cardiomyocytes in assays of 6–14 days. MIV-802 and its parent nucleoside are negative in micro-Ames up to 250 μg/plate and were negative in genotoxicity assays using mammalian cells. MIV-802 does not significantly affect mitochondrial DNA and RNA levels in cells. High levels of MIV-802-UTP (100-fold above its Ki against HCV NS5B polymerase) are rapidly formed in human hepatocytes, and decayed with a $t_{1/2}$ of 14 h. Hepatic MIV-802-UTP levels in dog 4 hours after MIV-802 dosing (50 mg/kg, p.o.) are 40-fold above the HCV NS5B polymerase Ki.
Conclusions: MIV-802 is a potent nucleotide analogue with excellent safety margins *in vitro* and delivers pharmacologically relevant amounts of UTP to human hepatocytes and dog liver. Its attractive preclinical profile supports progression of MIV-802 into clinical development for the future treatment of HCV infection in combination with other DAAs.

P0689

THE HEPATITIS C VIRUS CONTRIBUTES TO THE AGGRAVATION OF THE IMMUNOSUPPRESSIVE ENVIRONMENT BY INCREASING THE SUPPRESSIVE ACTIVITY OF NATURAL REGULATORY T CELLS

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Background and Aims: HCV infection is characterized by a high risk of chronicity. We have shown in a previous work that natural and induced human regulatory T cells play an important role in the progression of hepatitis C and are associated with the severity of viral recurrence after liver transplantation. However, nothing is known concerning the specific impact of HCV on these two regulatory T cell sub-populations. Our hypothesis is that HCV may promote the recruitment of regulatory T cells in the infected liver, and alter their phenotype and suppressive activity for the progression of liver pathogenesis.
Methods: Treg were isolated from the blood of healthy donors and infected *in vitro* with HCVcc/JFH-1. Internalization of viral proteins was highlighted by immunofluorescence and western blot (WB). Moreover, the expression of markers associated with phenotype and suppressive function of Treg cells was analyzed by Q-PCR, flow cytometry and WB. Proliferation and suppressive activity of infected Treg cells was analyzed by Thymidine of ^3H incorporation technique. In addition, the chemokines produced by hepatic cells and known to attract Treg were analysed by RT-QPCR.
Results: We showed that Treg infection with HCV significantly increases the expression of viral receptors and that the viral proteins are internalized into Treg. Interestingly, we also showed that viral infection raises the Treg anergy and promotes the recruitment of infected Treg cells by HCV-infected hepatic cells. In addition, HCV infection induced a significant increase in the expression of markers associated with Treg cells thus potentiating their "suppressive

phenotype". These results are correlated with the functional analysis of infected Treg cells, showing (i) a significant increase in the expression of markers associated with their suppressive activity (ii) a significant increase in the secretion of immunosuppressive cytokines and (iii) an increase in immunosuppressive function. Finally, we have shown that HCV promotes conversion of conventional T lymphocytes into induced T regulatory type 1 cells.
Conclusions: This study shows for the first time that HCV can be able to be internalized into human Treg cells and could promote Treg recruitment into the infected liver. Moreover, the fact that HCV can potentiate the suppressive function of nTreg and favor the induction of Tr1 may contribute to explain the mechanisms by which HCV escapes the immune system and promotes the progression of hepatitis C to cirrhosis and HCC

P0690
IFN-FREE THERAPY FOR CHRONIC HCV: TRANSCRIPTOMICS AND NK CELL ANALYSES

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Background and Aims: Chronic hepatitis C is a global health problem, resulting in liver failure, hepatocellular carcinoma and liver-related death. NK cells are players of the innate immune system and known to correlate to viral treatment response of HCV. In this study we investigate the effect of viral load decline with IFN-free direct acting antivirals (DAA) on T cells, natural killer (NK) cells and at the gene expression level in blood.
Methods: 12 patients with chronic HCV were treated with asunaprevir and daclatasvir and peripheral blood was analyzed at baseline, day 3, week 1, 2, 4, 8 and 12 during therapy. Virus-specific $CD8^+$ T cell frequency, blood transcriptomics and serum cytokines were analyzed. NK cell frequency, phenotype and ability to produce interferon-gamma (IFN-γ), perforin and granzyme were analyzed by flowcytometry. Sorted NK cells were cultured with K562 to analyze specific killing by 7-AAD. Inhibition of viral replication was determined by using $HuH7_{A2}$HCV replicon cell system.
Results: Our results show restoration of HCV-specific T cell frequency and fast down-regulation of interferon stimulating genes (ISGs) during IFN-free therapy. Serum IP-10, IFN-γ, IL-12p40 and IL-18 levels decreased early upon viral load decline. Surface expression of activation receptors NKp30, NKp46 and inhibitory receptor NKG2A on blood NK cells reduced during therapy. No effect of IFN-free therapy was observed on NK cell function, except for the expression of TRAIL on NK cells, which declined during therapy.
Conclusions: We demonstrate enhanced HCV-specific T cells during IFN-free DAA therapy and reduced ISG mRNA and protein. IFN-free therapy causes a reduction in NK cell stimulating cytokines in serum. In line, viral clearance corrects the NK cell phenotype by lowering the expression of activation and inhibitory signals and TRAIL.

P0691
KNOCKDOWN OF LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE 1 (LPCAT1) INCREASES BOTH THE SECRETION OF VERY-LOW-DENSITY LIPOPROTEINS (VLDL) AND THE INFECTIVITY OF HEPATITIS C VIRUS (HCV)

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Background and Aims: The hepatocyte is both the cell specialised in the secretion of VLDL, which are apolipoprotein (Apo) B-containing triacylglycerol (TG)-rich lipoproteins, and the primary replication site of HCV. Unique to this virus is its intimate link with the host lipid metabolism. The most infectious HCV particles are those of lowest density due to their association with TG-rich lipoproteins. Moreover, HCV-infected patients frequently have hepatic steatosis, i.e., accumulation of TG in cytosolic lipid droplets (LD), which store neutral lipids within a monolayer of phospholipids, mainly phosphatidylcholine. We recently showed that HCV downregulates LPCAT1, about which little is known except that it can synthesize phosphatidylcholine on the LD surface. Here we investigated the role of LPCAT1 in the lipid metabolism of hepatocytes and in HCV infectious cycle.
Methods: Huh-7.5.1 cells and primary human hepatocytes (PHH) were infected with JFH1. Knockdown of LPCAT1 was achieved by RNA interference technology. Cells were monitored for LD size (immunofluorescence) and TG level (enzymatic assay). Culture supernatants were probed for the secretion of ApoB (ELISA) and lipids (metabolic labelling using C^{14}-oleic acid), and for HCV production (viral load test) and infectivity (titration). The density of viral particles was assessed by isopycnic ultracentrifugation.
Results: LPCAT1 knockdown in either naïve or infected Huh-7.5.1 cells increased the mean size of LD and the intracellular level of TG in the steady state, thus causing steatosis. It also increased the secretion of both ApoB and newly synthesized TG, consistent with an impact on VLDL biosynthesis. In infected Huh-7.5.1 cells or PHH, LPCAT1 knockdown increased production of the viral particles with highest infectivity and lowest density, consistent with their enrichment in TG. Finally, the excess of ApoB secreted from infected PHH was found at the density of the viral particles, compatible with the formation of hybrid structures between HCV and VLDL.
Conclusions: LPCAT1, a LD-associated protein thought to be a phospholipid membrane remodelling enzyme, has a role in the metabolism of TG. Its downregulation by HCV appears as a viral strategy to hijack the VLDL biosynthesis pathway for the production of highly infectious hybrid particles. Targeting the LPCAT1-regulated lipid metabolism pathway may thus represent an attractive therapeutic approach able to reduce both the viral titre and hepatic steatosis.
FB and ML, or ARR and SD: equal contribution.

P0692
PKC/AP-1 SIGNALING DRIVES TRANSCRIPTION OF INTERFERON-STIMULATED GENES AND EXERTS POTENT ANTIVIRAL ACTIVITY AGAINST HEPATITIS C AND E VIRUSES

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Background and Aims: Protein kinases are enzymes that modifies proteins by chemically adding phosphate groups (phosphorylation)

and are considered pivotal regulators of almost all cellular processes. Strikingly, however, their involvement in viral infection is less well defined. This study aimed to identify kinase-mediated signaling pathways modulating hepatitis C (HCV) and E (HEV) virus infections and to explore the feasibility of modulators of kinase activity as antiviral targets.
Methods: Huh7 cell line harboring subgenomic HEV luciferase reporter, full-length HEV infectious genome or subgenomic HCV were used.
Results: Screening of a library containing 132 kinase inhibitors identified a Protein Kinase C (PKC) inhibitor that significantly promotes HEV infection. Consistently, RNAi mediated knockdown of PKCα, a key member of the PKC family, results in a significant 7-fold increase in HEV RNA levels. Conversely, overexpression of an active form of PKCα inhibited HEV RNA production by 77.5%. Phorbol-12-myristate-13-acetate (PMA) is a well-established PKC activator. As expected, treatment with PMA significantly inhibits both HCV (by 50%) and HEV (by 68%) replication. Combining PMA with ribavirin, but not interferon-alpha exerted additive antiviral activity, suggesting a possible convergence of PKC and interferon signaling. Indeed, in our experimental system PMA potently transactivates Interferon stimulated response elements (ISRE) and induces expression of Interferon stimulated genes (ISGs) independent of the canonical elements of the interferon signaling, including IRF9 and JAK1. Knockdown of c-fos, a key component of the AP1 complex that is downstream of PKC, completely abolishes the capacity of PMA to activate the ISRE and to induce ISGs. Bioinformatical analysis reveals a consensus nucleotide sequence within the ISRE and AP1 DNA binding motifs. Mutagenesis of this homologous region within the ISRE motif removes the capacity of PMA to transactivate the ISRE, confirming that the AP1 complex directly driving the transcription of ISRE to induce ISG expression.
Conclusions: Together with canonical interferon signaling, the PKC/AP1 signaling converges on ISRE-containing promotors to induce the expression of antiviral ISGs. Thus PKC/AP1 signaling a novel indispensable branch of cell-autonomous antiviral signaling constraining HCV and HEV infection.

P0693
THE TUMOR SUPPRESSOR PML MEDIATES EVEROLIMUS-INDUCED CHANGES OF THE HCV REPLICATION ACTIVITY
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Background and Aims: Successful liver transplantation (LT) due to hepatitis C virus infection is often followed by a deleterious course of HCV reinfection of the liver graft. New antiviral treatment options give hope for improvement the course of disease and prognosis. Nevertheless, the influence of immunosuppression on HCV replication and reinfection, particularly of mTor inhibitors, is not clarified yet. A previously described interaction of the tumor suppressor PML with the mTor signaling cascade as well as findings that the HCV interacts with PML suggest some relevance of the tumor suppressor in that context. The aim of our study is to analyze the influence of everolimus (EVR) on HCV replication activity *in vitro* in the context of the underlying molecular mechanisms with focus on the role of PML.
Methods: Subgenomic HCV replicons for genotype (GT) 1a and 2b were treated with different immunosuppressive substances. For detection of cell viability FACS-based CFSE analyses as well as MTT assays were performed. PML expression was silenced employing shRNA constructs. Protein and mRNA expression was quantified by qrt-PCR and western blot analysis. Gene array analysis was employed to reveal underlying molecular mechanisms.
Results: mTor-Inhibitors and calcineurin inhibitors (CNIs) have different effects on HCV replication activity. Therapeutic doses of EVR impair HCV replication activity of GT2a up to 50% (P≤0.001). In contrast, replication activity of HCV GT1b is increased by more than 50% (P≤0.001) under the influence of EVR. Interestingly, in absence of PML the effect of EVR on HCV replication activity is nearly abrogated. Even without EVR treatment, HCV replication activity is impaired in the absence of PML while transfection of PML-deficient replicon-containing cells with different PML isoforms results in a recovery of HCV replication activity. Gene array analyses of Huh7.5 replicon cells reveal a divergent expression pattern upon treatment with CNIs or mTor-Inhibitors and, thus, interesting candidate genes as further key components in EVR-induced regulation of HCV replication activity.
Conclusions: We demonstrate a genotype-dependent influence of EVR on HCV replication activity *in vitro*. Our results demonstrate an interplay between the expression of PML and the EVR-induced reduction of HCV replication activity. The results of this study suggest that the choice of immunosuppressive therapy after LT may significantly influence the progress of HCV reinfection.

P0694
17BETA-ESTRADIOL INHIBITS HCV INFECTION VIA BINDING TO ITS RECEPTOR
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Background and Aims: Hormonal factors may play a role in controlling hepatitis C virus (HCV) infection. In fact, spontaneous HCV clearance is more common among premenopausal women than in men and, when infection persists, histologic progression of chronic hepatitis is slower in pre-menopausal women in comparison to postmenopausal women and men. Based on these premises, we aimed to evaluate sex hormones as inhibitors of HCV replication.
Methods: Huh-7.5 cells infected with the JFH-1 virus (genotype 2a) were exposed to one each among the following hormones: dehydroepiandrosterone (DHEA), testosterone, progesterone and 17β-estradiol (the latter in the presence/absence of the estradiol receptor antagonist fulvestrant). Based on the inhibition of viral replication, dose-response curves covering the physiological range were established allowing calculation of IC50 values. In model A, hormones were added 3 hours post-infection. In model B, Huh-7.5 cells were incubated with hormones for 1 h before being infected; 3 h later, the inoculum was replaced with fresh medium. In model C, a 16 h pre-incubation 17β-estradiol preceded replacement with fresh medium, followed by infection.

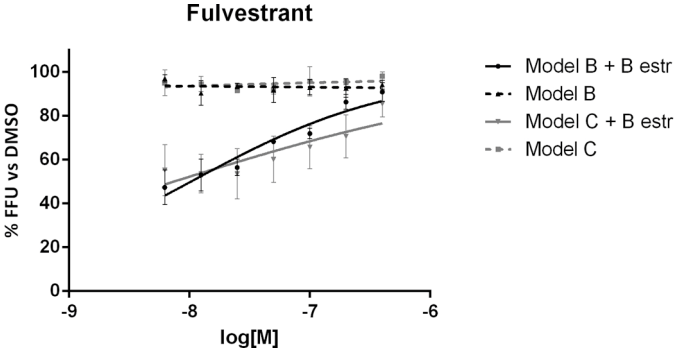

Figure: %FFU indicates the percentage of focus forming unit reduction (FFU); the horizontal line represents the IC50 values.

Results: Progesterone and testosterone did not show any inhibitory effect on viral replication at the concentrations tested for both model A or B. DHEA exhibited only a partial antiviral effect in both model A and B (39%). In contrast, in model A 17β-estradiol inhibited viral replication up to 45%, allowing to estimate IC50 = 490 nM. Moreover, the inhibitory effect of 17β-estradiol reached 67% in model B and 64% in model C, with IC50 values of 140 and 160 nM, respectively. Although fulvestrant did not exhibit any appreciable effect on viral replication, in its presence the inhibition exerted by 17β-estradiol was reverted in a dose-dependent manner (Figure).
Conclusions: In vitro, 17β-estradiol is able to block HCV infection, likely by modulation of intracellular pathways following binding to the estradiol receptor, whose activation leads to an antiviral state.

P0695
TRIPLE THERAPY FOR CHRONIC HCV PATIENTS INDUCES EARLY ACTIVATION OF INTRAHEPATIC NK CELLS
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Background and Aims: Previous studies have correlated therapy outcome in HCV to the expression of various markers on NK cells, including inhibitory killer immunoglobulin-like receptors CD158a and CD158b as well as natural cytotoxicity receptor NKp46 and activating receptor CD69. This study is the first to investigate intrahepatic NK cells in HCV patients during triple therapy with telaprevir. By collecting multiple liver fine-needle aspirate biopsies (FNABs) we have the unique opportunity to investigate liver NK cells during therapy-induced viral load reduction.
Methods: 10 chronic HCV patients were treated with peg-interferon/ ribavirin and telaprevir according to prescription protocol and all responded well to therapy. Intrahepatic leukocytes were obtained via aspirate biopsy at baseline, 6-hours, 1-week, and 12-weeks of treatment. Peripheral blood was collected at various time-points and peripheral blood mononuclear cells (PBMC) were obtained via ficoll separation. Expression levels of CD158a, CD158b, NKG2A, NKG2D, NKp46, and CD69 were analysed on $CD56^+$ $CD3^-$ NK cells by flowcytometry.
Results: At baseline, liver NK cells displayed a more activated phenotype compared to blood based on the expression of CD69 and NKG2D ($p = 0.001$ and $p = 0.05$ respectively). Expression of NK cells receptors in liver of individual patients highly correlated with frequencies in blood (all $p < 0.05$). Sampling of the liver 6 hours after start of therapy showed no evidence for an immediate effect of triple therapy on liver NK cells, since no modulation of NK cell phenotype was observed. After 1 week therapy, paired sampling of the liver showed a significant increase in activating receptors NKp46 and NKG2D ($p = 0.02$ and $p = 0.05$ respectively) and a decrease in inhibitory receptor NKG2A ($p = 0.03$) on total NK cells, indicating a more activated phenotype. Interestingly, after 12 weeks of therapy, expression of NKG2D and NKp46 on liver NK cells returned to baseline levels, while expression of NKG2A significantly increased compared to all time-points ($p = 0.03$).
Conclusions: Our results demonstrate that liver NK cells are more activated compared to blood. Our data suggest an active role for liver NK cells during triple therapy with dynamic alterations of NKG2A, NKG2D and NKp46 levels on liver NK cells of chronic HCV patients during the early phase of triple therapy.

P0696
PROTEASOME SUBUNIT ALPHA TYPE-6 REGULATES THE EXPRESSION OF PROVIRAL HOST GENES IN HEPATITIS C VIRUS INFECTION
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Background and Aims: The interferon stimulated gene 15 (ISG15) plays an important role in the pathogenesis of hepatitis C virus (HCV) infection. ISG15-regulated proteins have previously been identified that putatively affect this proviral interaction. The present observational study aimed to elucidate the relation between ISG15 and these host factors during HCV infection.
Methods: Proteomic and transcriptomic analyses were performed using liver samples of HCV-infected ($n = 54$), HBV-infected patients ($n = 23$) and uninfected controls ($n = 10$). Primary human hepatocytes (PHH) were treated with toll-like receptor ligands, interferons and kinase inhibitors. Expression of *ISG15* and proteasome subunit alpha type-6 (*PSMA6*) was suppressed in subgenomic HCV replicon cell lines using specific siRNAs.
Results: Comparison of hepatic expression patterns revealed significantly increased signals for ISG15, IFIT1 (interferon-induced protein with tetratricopeptide repeats 1), HNRNPK (Heterogeneous nuclear ribonucleoprotein K) and PSMA6 on the protein level as well as *ISGs* and *PSMA6* on the mRNA level in HCV-infected patients. In contrast to *ISGs*, *PSMA6* expression occurred independent of HCV load and genotype. Expression of *ISG15* and *PSMA6* was induced by poly(I:C), depending on interferon regulatory factor 3 activation and phosphoinositide 3-kinase/AKT signalling, respectively. Interestingly, suppression of *PSMA6* led to significant induction of *ISG15* expression, thus combined knockdown of both genes abrogated the antiviral effect induced by the separate suppression of *ISG15*.
Conclusions: PSMA6 is up-regulated during viral hepatitis and functions as a negative regulator for ISG15, a proviral factor in the pathogenesis of HCV infection. These findings suggest that the proteasome might affect the enigmatic interaction between ISG15 and HCV.

P0697
INTERPLAY BETWEEN VIRUS-SPECIFIC AND APOPTOTIC EPITOPE-SPECIFIC CD8+ T CELLS IN CHRONIC HEPATITIS C VIRUS
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Background and Aims: $CD8^+$ T cells specific to caspase-cleaved antigens derived from apoptotic T cells (apoptotic epitopes [AE]) represent a principal player of chronic immune activation (CIA), known to amplify immunopathology in various inflammatory conditions. The purpose of this study was to investigate the interplay among HCV-specific $CD8^+$T cells, AE-specific $CD8^+$T cells, and disease activity in chronic hepatitis C virus (cHCV) infection.
Methods: We enumerate both HCV- and AE-specific $CD8^+$T cells from PBMCs of patients and donors by MHC-I

dextramers complexed to the relevant peptides. We analyzed cytokine production in dextramer$^+$CD8$^+$ T cells in response to peptide stimulation by intracellular staining. We characterized dextramer$^+$CD8$^+$ T cell phenotype with surface and nuclear markers. mRNA levels of IFN stimulated gene (MxA, PKR, ISG15) were analysed done by Quantitative PCR assays (SYBR Green technology). The genotyping for the *IL28B* rsl2979860 SNP was performed with LightMix® *in-vitro* diagnostics Kit IL28B.

Results: Both HCV- and AE-specific CD8$^+$ T cells exhibited parallel frequencies and correlated with transaminase levels and liver fibrosis score, respectively, supporting a possible disparity in sustaining liver damage (direct hepatocyte killing *vs.* by-stander inflammation). Regardless, both AE- and HCV-specific CD8$^+$ T cells displayed unusual functional properties, on the basis of both the expression of a functional phenotype (T-bet^{high}/Eomes$^+$/PD-1^{low}) and the capacity to produce IFN-g by a high percentage of them, particularly when they migrated into the inflamed liver. Importantly, AE-specific (but not the HCV-specific) CD8$^+$ T cells correlated with the level of some IFN type I-stimulated gene transcripts in PBMCs.

Conclusions: Our data strongly indicate that these cells are not terminally dysfunctional and may contribute to maintain chronic active disease. Accordingly, the evidence that only a minority of them expressed a "dysfunctional phenotype" (i.e., PD-1$^+$, PD-1$^+$/T-bet$^-$/Eomes$^+$, or Tbet^{low}/Eomes$^+$ cells), and that the immunoregulatory PD-1/PD-L1 pathway was operative *in vitro*, suggests that the latter likely contributes to limit immunopathology, rather than to induce severe T cell dysfunction. The finding that AE-specific (but not the HCV-specific) CD8$^+$ T cells correlated with the level of IFN type I-stimulated gene transcripts in PBMCs suggest that the chronic IFN-I signaling contributes in establishing CIA and disease progression by modulating AE-specific CD8$^+$ T cell responses in cHCV infection.

P0698
QUERCETIN MODIFIES LIPID DROPLET MORPHOLOGY AND IMPAIRS HEPATITIS C VIRAL LIFE-CYCLE STEPS FROM ASSEMBLY TO REPLICATION STEPS

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Background and Aims: Hepatitis C virus (HCV) life cycle can be divided into several steps: (i) entry of viral particles, (ii) translation of the viral proteins, (iii) replication of the viral genome, a step which requires the activity of HCV non structural proteins including the protease NS3, and (iv) assembly of new viral particles, a step which requires the localization of HCV core and NS5A protein to lipid droplets mediated by the host diacylglycerol acyltransferase type 1 (DGAT1). Quercetin, a bioflavonoid, seems to prevent the localization of HCV core protein to lipid droplets and to inhibit HCV replication (Rojas et al., AASLD 2013). Here we aimed at evaluating the potential of quercetin as antiviral drug and further defining its mechanism(s) of action on the different steps of HCV life cycle.

Methods: To reproduce the complete HCV life cycle, Huh7.5 cells and primary hepatocyte were infected with JFH1and subsequently treated with doses of quercetin. (i) Replication of HCV genome was assessed by measuring the intracellular levels of negative-strand HCV RNA by qRT-PCR. (ii) Production of infectious virus was assessed by measuring the infectivity titers in filtered culture supernatants with focus-formation assay and by COBAS® TaqMan® HCV Test v2.0. (iii) NS3 protease activity *in vitro* was measured using a comercial Kit SensoLyte® 520 HCV Protease Assay. (iv) DGAT activity was analyzed using the protocol previously described by McFie and coll. (2011) in Huh7.5 cells infected by JFH1.

Results: Infectivity assay in Huh7.5.1 and primary hepatocytes were IC50: 37.83 and 23.63 μM respectively. At 50μM HCV-RNA levels decreased (Huh7.5.1: 39% and PHH: 24%). The amount of HCV-RNA (evaluated by the quantity of viral RNA produced in the supernatant) was decreased as well by 60%±26.7 ($p < 0.05$) compared to the supernatant from Huh7.5 infected by JFH.1 (1MOI). *In vitro* NS3 activity was inhibited by quercetin by 45.40%±1.15 RFU ($p < 0.001$) compared to the vehicle, DMSO (no inhibition). DGAT enzyme activity in infected cells was increased relative to non-infected cells (*fold induction* 2.29±0.23 $p < 0.01$). However, this increase was significantly inhibited by treatment with quercetin [63.5±2.9% ($p < 0.01$)]

Conclusions: In the current study, quercetin was observed to inhibit DGAT activity, to decrease NS3 activity, as such, resulting in impairment of viral infectivity and replication. Thus, the antiviral activity of this flavonoid is promising and mediated through several viral and host mechanisms.

P0699
EPIGENETIC HARNESSING OF HCV VIA MODULATING THE LIPID DROPLET-PROTEINS, ADRP AND TIP47, IN HCV CELL MODELS

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Background and Aims: Host cell lipid droplets (LDs), along with their associated perilipin protein family (PAT), were reported to promote hepatitis C virus (HCV) life cycle. The regulation of PAT proteins expression and subsequently cellular LDs by microRNAs (miRNAs) has never been investigated in HCV cell models. Therefore, this study aimed at identifying miRNAs that target and modulate the hepatic PAT proteins, adipose differentiation related protein (ADRP) and Tail interacting protein of 47 kDa (TIP47), and subsequently regulate LDs and HCV infection.

Methods: Bioinformatics analysis was performed to predict miRNAs that target ADRP and TIP47. Expression profile of identified miRNAs and their targets was examined in 21 HCV-infected liver biopsies and 9 healthy donor liver tissues obtained during liver transplantation as well as in JFH-I infected compared to naïve Huh7 cells using qRT-PCR. JFH-1 infected cells were transfected with identified miRNA mimics or antagomirs or siRNAs against ADRP or TIP47 followed by oleic acid treatment to induce LD formation to study their effect on ADRP and TIP47 expression as well as on intracellular LD and HCV RNA levels. LDs were stained with oil-red-O and visualized using immunofluorescence microscopy. HCV RNA was quantified using qRT-PCR.

Results: Bioinformatics revealed that ADRP is a potential target of miR-148a and TIP47 potentially targeted by miR-148a and miR-30a. Expression profiling showed that miR-148a, miR-30a and ADRP were downregulated ($p = 0.0216$, $p = 0.0041$ and $p = 0.0261$), while TIP47 was upregulated ($p = 0.0489$) in patients compared to controls. Infection of Huh7 cells with JFH-I suppressed ADRP and induced TIP47 expression ($p = 0.0167$, $p = 0.0167$), suggesting that the alteration in PAT protein expression is due to the viral infection. Mimicking of both miRNAs significantly induced ADRP ($p = 0.0103$, $p = 0.02$), while they suppressed TIP47 expression ($p = 0.0488$, $p = 0.0262$). Cellular LD content was reduced in miR-148a, miR-30a mimicked and TIP47 knockdown cells. HCV RNA was markedly decreased upon mimicking with miR-148a and miR-30a

by a mean of 36% and 42.65%, respectively, and in TIP47 knockdown cells by 42.9% but was not affected by ADRP knockdown.
Conclusions: In conclusion this study shows for the first time that HCV infection deregulates hepatic ADRP and TIP47 expression in HCV infected patients and cell lines. Forcing the expression of miR-148a and miR-30a, induces the expression of ADRP and suppresses TIP47, decreases cellular LDs and subsequently suppresses HCV infection.

P0700
IMMUNOLOGICAL RATHER THAN VIROLOGICAL RESPONSE TO HAART IS ASSOCIATED WITH IMPROVED ANTI-HCV NK CELL ACTIVITY IN HIV PATIENTS

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Background and Aims: Co-infection with the hepatitis C virus (HCV) is a major cause of morbidity and mortality in HIV(+) patients.
Natural killer (NK) cells are considered to play an important role in the immunobiology of HCV-infection, as they have been shown to exert anti-viral functions. Interestingly, in HIV/HCV co-infected individuals anti-HCV activity of NK cells is impaired. However, it is unclear, how HIV-infection affects NK cell activity. Thus, we analyzed the anti-HCV activity of NK cells in HIV mono-infected patients.
Methods: 40 HIV-infected patients, including 21 individuals who were HIV RNA(−) under HAART and 19 treatment-naïve HIV RNA(+) patients, as well as 20 healthy HIV(−)/HCV(−) controls were enrolled into this study. Anti-viral activity of total PBMCs was studied using the HUH7-HCV-replicon system. IFN-γ secretion of NK cells was analyzed in the presence/absence of CD3/CD28 stimulated $CD4^+$ T cells
Results: Compared to healthy individuals we found PBMCs from HIV-RNA(+) patients to display significantly impaired anti-HCV activity. Of note, HIV-RNA(−) patients under effective HAART displayed a similar dys-regulation of anti-viral activity. Accordingly, we found IFN-γ production following co-incubation with replicon cells to be significantly impaired in HIV patients, irrespective of HAART. However, when patients were stratified according to $CD4^+$ T cell counts we found high $CD4^+$ T cell numbers (>400/μl) to be associated with strong anti-HCV activity. In line with this finding we observed IFN-γ production of NK cells to be significantly correlated with frequency of $CD4^+$ T cells. In co-culture experiments we found that $CD4^+$ T cells effectively stimulate IFN-γ production of NK cells.
Conclusions: Here, we show that HIV-infection caused an impaired anti-HCV activity and IFN-γ secretion of NK cells in HIV-RNA(+) and HIV-RNA(−) patients. However, in contrast to viral loads we found $CD4^+$ T cell frequency to be correlated with anti-HCV activity. Therefore, our findings indicate that immunological rather than virological response to HAART might be associated with an improved anti-HCV NK cell activity in HIV(+) patients.

P0701
NEXT GENERATION SEQUENCING REVEALS DIFFERENTIAL EXPRESSION PROFILES OF HEPATIC MICRORNAS IN CHRONIC HEPATITIS C PATIENTS

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Background and Aims: Hepatitis C virus (HCV) is a major cause of chronic liver disease worldwide. MicroRNAs (miRNAs) are small, non-coding RNA molecules of 20–22 nucleotides that that act post-transcriptionally to regulate gene expression. Previous studies have demonstrated association of several miRNAs to the HCV disease progression and implicated as potential new therapeutic targets and biomarkers. However, the data from such studies is restricted to analysis of limited miRNAs. Recently, we have reported next generation sequencing (NGS) based profiling of differentially expressed miRNAs (DE-miRNAs) between chronic hepatitis C (CHC) and healthy subjects. In the present study, we report deep sequencing analysis of DE-miRNAs between different groups of CHC patients grouped according to the response to interferon therapy.
Methods: A total of eight pre-treatment percutaneous liver biopsy specimens from the CHC patients (genotype 3) [sustained virological responders (SVR; n = 4) and non-responders (NR; n = 4)] were selected according to the end treatment response (ETR) to interferon plus ribavirin therapy. The libraries were sequenced on HiSeq2000. DE-miRNAs were identified by edgeR package. Selected DE-miRNAs were also validated by using real time PCR in an independent cohort of CHC patients (n = 120).
Results: We identified 29, 14, and 12 DE-miRNAs between SVR: NR, SVR: R and NR: R patients groups (-1.5 < fold change >1.5; $P < 0.05$) respectively (Figure 1). DE-miRNAs from each group was then profiled in cohort group and found to be in agreement with NGS findings. The diagnostic and prognostic potential of DE-miRNAs in different groups was assessed by ROC curves and regression analyses. Furthermore, in order to assess the effect of DE-miRNAs on the host's genes expression and biological functions, expression profiles of liver-specific genes in CHC patients were obtained and an integrated miRNA-mRNA interaction, ontology and pathway analysis was performed. This showed that DE-miRNAs in SVR and NR groups target several genes associated with interferon-α, TNF, JAK-STAT, immune response and lipid metabolism pathways.

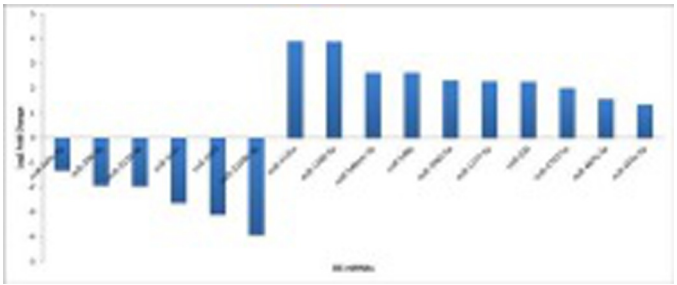

Figure 1.

Conclusions: In summary, we show that liver-expressing miRNAs show significantly different expression patterns among CHC patients when classified on the basis of ETR. The findings highlight that DE-miRNAs have the potential to serve as novel biomarkers in CHC patients. Moreover, the miRNA-mRNA interactions and pathway analysis show that DE-miRNAs can also serve as attractive therapeutic targets in CHC patients.

P0702

CC IL28B GENOTYPE IS ASSOCIATED WITH HIGH LIVER NECROINFLAMMATION AND INCREASED EXPRESSION OF TH1 CYTOKINES (CYK) AND CHEMOKINES (CHK), BUT ALSO HIGHER TH2 CYK WHICH MAY DRIVE LOW ISG EXPRESSION

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Background and Aims: IL28B genotype (gt) and ISG expression predict IFN response, but the mechanisms are poorly understood. We hypothesized that ChK and Th1 CyK that mediate inflammatory responses higher in C/C *IL28B* genotype which leads to upregulation of Th2 CyK resulting in lower intrahepatic ISG expression. We also hypothesize that there is an early and more potent induction of ChK/CyK in C/C *IL28B* genotype patients which predicts for HCV clearance. We performed a detailed study of the relationship between *IL28B* genotype and expression in of a panel of ChK and relevant Th1/Th2 CyK in HCV patients (pts) with paired plasma and liver. We also compared on-treatment changes in plasma ChK/CyK during the first 4 weeks of pegIFN+RBV dual therapy (PR) in a subset of HCV pts.

Methods: HCV-1 pts with paired plasma and liver tissue were included. IL28B gt (rs12979860) was determined. Liver necroinflammatory activity was scored using METAVIR activity score. Liver expression of ChK (CCL2/CXCL9/CXCL10) and CyK (IL1b/IL6/IL8/IL10/IL12/TNFa) were measured (rtPCR). Plasma CyK/ChK were measured by cytometric bead array (BD). Plasma was collected at days 0, 1, 7, 14, 28 for detailed analysis of IFN-induced ChK/CyKs in a subset of pts treated with PEG+RBV. ChK/CyK expression were correlated with liver necroinflammation, IL28B gt and treatment outcome.

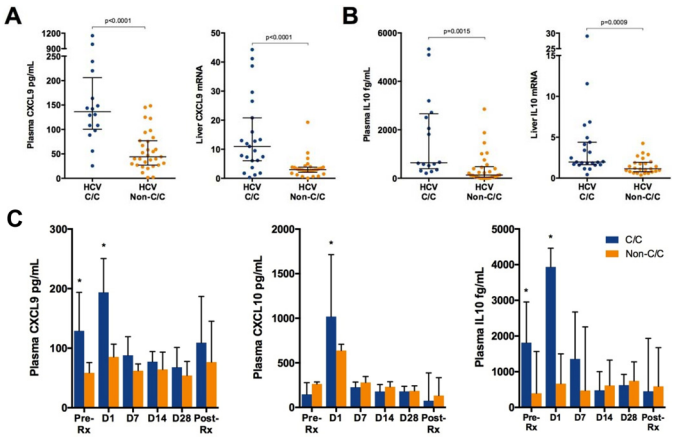

Figure 1. (A) Plasma and liver CXCL9 levels according to *IL28B* genotype. (B) Plasma and liver IL10 levels according to *IL28B* genotype. (C) On-treatment CXCL9, CXCL10 and IL10 according to *IL28B* genotype. *Significant p-value.

Results: 47 pts with paired liver/plasma samples were included: 34% CC IL28B gt. Necroinflammation was significantly higher in CC IL28B with 81% having A2–3 METAVIR activity (vs 32% for non-CC pts, p = 0.001). ALT was also significant higher (109 vs 57 in CC vs non-CC, p = 0.036). Plasma and liver Th1 CyK and CXCL9 levels were significantly higher in CC IL28B gt patients (Figure 1a). Plasma and liver IL10 (Th2 CyK) were also significantly higher in CC pts (Figure 1b). In treated patients (n = 19), an early and rapid increase in plasma CXCL9, CXCL10, and IL10 levels were observed in CC pts and those with subsequent SVR, compared to non-CC pts and pts who did not achieve an SVR (Figure 1c). Peak CyK and ChK levels peaked at day 1 of PR therapy.

Conclusions: The data identify an important role for the chemokine CXCL9 in mediating inflammation in the HCV liver, which is strongly associated with IL28B gt. IL10 upregulation was also observed in CC pts which may represent a secondary response to Th1 upregulation which then lowers liver ISG expression. Interferon treatment induces a potent and early ChK and IL10 response, which is associated with IL28B gt and predicts for HCV clearance.

P0703

PRE-TREATMENT RIBAVIRIN SENSITIVITY CORRELATES WITH TREATMENT OUTCOME IN GENOTYPE 3 HCV

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Background and Aims: New therapies for chronic HCV infection have substantially increased rates of sustained virological response (SVR). However relapse after therapy remains a problem, especially in patients with cirrhosis. We have developed a novel capture-fusion assay to study patient-derived HCV (1). Here we demonstrate that this assay can identify pre-treatment sofosbuvir (SOF), interferon (IFN) and ribavirin (RBV) sensitivity in patients with G3 HCV, and RBV sensitivity correlates with treatment outcome.

Methods: Archived pre-treatment sera were obtained from 10 G3 patients treated with pegIFN/RBV, 4 with SVR and 6 who relapsed, and from 4 G3 patients treated with SOF/RBV, 3 with SVR and 1 who relapsed. THP-1 cells were exposed to donor serum, fused with Huh7.5 cells and treated with SOF, IFN or RBV before qPCR assessment of HCV replication. Results are given as mean ± sem and p values were calculated using Mann Whitney U test.

Results: No difference in pre-treatment IFN sensitivity was seen between patients with SVR and those who relapsed after pegIFN/RBV (IFN IC_{50} 0.61±0.11 IU/mL for patients with SVR versus 0.55±0.09 IU/mL for relapse, p = 0.61). However pre-treatment isolates from patients with SVR were significantly more sensitive to RBV than those from patients who relapsed (ribavirin IC_{50} 0.62±0.05 μM for patients with SVR versus 1.25±0.13 μM for relapse, p = 0.01). Amongst patients treated with SOF/RBV, pre-treatment SOF sensitivity was similar between patients with SVR and relapse (SOF IC_{50} 0.036±0.016 μM for SVR versus 0.023 μM for the patient who relapsed). However pre-treatment RBV sensitivity appeared greater in patients with SVR than relapse (RBV IC_{50} 0.377±0.037 μM for SVR versus 1.049 μM for the patient who relapsed).

Conclusions: These data confirm the value of the capture-fusion assay in predicting response to treatment of chronic HCV infection. We found a reduced response to RBV in patients with G3 HCV who relapsed compared to patients who achieved SVR following both IFN- and SOF-based therapy, suggesting that RBV sensitivity may be an important determinant of treatment response in this genotype. Further studies to confirm this observation, including viral sequencing and phenotyping in chimeric replicons, are underway.

Reference(s)

[1] Cunningham ME, Javaid A, Waters J, Davidson-Wright J, Wong JL, Jones M, Foster GR. Development and validation of a 'capture-fusion' model to study drug sensitivity of patient-derived hepatitis C. Hepatology 2014.

P0704

DEEP-SEQUENCING ANALYSIS DEMONSTRATES THE PERSISTENCE OF THE PRE-TRANSPLANT HCV DOMINANT VARIANT WITHIN A MORE HOMOGENEOUS QUASISPECIES AFTER LIVER TRANSPLANTATION IN CHOLESTATIC HEPATITIS C PATIENTS

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Background and Aims: Little is known about the pathogenic mechanisms implicated in cholestatic hepatitis C (CH), a severe form of hepatitis C (HCV) recurrence leading to high graft loss rates early after liver transplantation (LT). Classically, host failure to mount specific T-cell responses against HCV (leading to uncontrolled HCV replication and hepatocyte damage) has been implicated in the pathogenesis of CH. However, a direct role of HCV is not well established. The aim of this study was to analyze, for the first time, the pattern of HCV quasispecies evolution in patients with CH (compared to a control group) by ultra-deep pyrosequencing (UDPS).
Methods: 13 HCV-infected liver transplant recipients with CH (Wiesner et al., Liver Transpl 2003) and 10 patients with mild HCV recurrence (control group) have been analyzed so far. A serum sample obtained at the time of LT and a second sample obtained 1–3 months after transplantation (acute hepatitis phase) were analyzed for each patient. UDPS of a NS5B fragment (339 nucleotides) was performed using the 454 GS Junior platform (Roche).
Results: All 23 patients were infected with genotype 1 (87% G1b). Donor age ($p = 0.007$), recipient age ($p = 0.022$) and viral load after LT ($p < 0.001$) were significantly higher in patients with CH compared to controls. The mean number of reads per sample was 6146 (range 2491–25820). NS5B quasispecies complexity before LT was similar in CH and control group ($p = 0.396$). On the contrary, genetic diversity and mutation frequency of NS5B quasispecies decreased significantly after LT in patients with CH ($p = 0.013$ and $p = 0.013$), but not in the control group ($p = 0.069$ and $p = 0.327$). Interestingly, phylogenetic analysis showed that the major HCV variant pre-LT successfully propagated and remained as the major variant after LT in 64% of patients with CH, but only in 12% of controls ($p = 0.026$).
Conclusions: Using UDPS, a marked homogenization of HCV quasispecies was demonstrated in HCV-infected patients who developed CH after LT. In addition, in most CH patients, the major HCV strain before LT remained as dominant after LT. The latter suggests that, in the context of immunosuppresion, the propagation of more fitted viral strains could play a role in the pathogenesis of CH.

P0705

ANTIVIRAL ACTIVITY OF HUMAN NON-PARENCHYMAL LIVER CELLS IS RESTRICTED TO TOLL-LIKE RECEPTOR 3 AND INVOLVES TYPE I INTERFERONS

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Background and Aims: Chronic hepatitis C infection is associated with increased expression of interferon-sensitive genes (ISGs) in the liver. However, the role of non-parenchymal liver cells (NPC) in the defence against hepatitis C virus (HCV) infection is not well understood. Aim of the study was to characterize primary human Kupffer cells (KC), liver sinusoidal endothelial cells (LSEC) and hepatic stellate cells (HSC), isolated from a single liver tissue, to analyse the toll-like receptor (TLR) signalling and antiviral capacities against HCV.
Methods: NPC were isolated after two step collagenase perfusion of liver resections ($n = 25$), using low speed centrifugation, density centrifugation and MACS. Cell purities were controlled by immunofluorescence staining of cell type-specific markers: CD68, CD146 and α-smooth muscle actin (α-SMA). The functional activity of NPC was determined by cell type-specific assays. Cultured cells were stimulated with TLR ligands for 6–24 h, RNA was extracted and analysed by qRT-PCR and supernatants were collected to analyse the antiviral activity by co-culturing supernatants with HCV replicon system con1 and neutralizing antibodies against type I and III interferon (IFN) receptors. In addition, cells were stimulated with TLR ligands for 90min and cell lysates were examined for the activation of the interferon regulatory factor 3 (IRF3).
Results: Immunofluorescence analysis indicated high purities of KC (94.4±2.0%), LSEC (98.1±0.3%) and HSC (96.6±0.9%) with retained physiological activity *in vitro*. NPC secreted inflammatory cytokines (e. g. IL-6), in response to TLR1–9 stimulation in a cell type-specific manner. However, only supernatants of TLR3-activated NPC mediated an antiviral activity against HCV. Poly I:C treatment led to the activation of IRF3, an important transcription factor for IFNs. Neutralization experiments revealed that the antiviral effect was mediated by type I IFNs.
Conclusions: NPC were isolated in high cell yield and purity. Cell populations retained specific morphologies and physiological activity *in vitro*. The presented cell isolation technique is a valuable tool with high potential to investigate liver function and disease. Here, NPC responded to TLR ligands by producing inflammatory cytokines. An antiviral effect was restricted to TLR3-activated NPC and seemed to be mediated by type I IFNs. These findings shed new light on the impact of NPC in the pathogenesis of HCV.

P0706

INTRAHEPATIC EXPRESSION OF ABCA1 IN PATIENTS WITH CHRONIC HEPATITIS C

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Background and Aims: ATP binding cassette-A1 (ABCA1) is an integral transmembrane protein, essential for cholesterol and phospholipid homeostasis, with anti-atherogenic and anti-tumoral properties. It has been recently shown that the up-regulation of ABCA1 gene expression impairs hepatitis C virus HCV infection "in vitro", acting on virus protein mediated-host cell fusion (PLoS One 2014; 9(3): e9214). We wished to confirm these results "in vivo", hypothesizing that differences in ABCA1 expression may affect viral load and/or affect the progression of liver disease.
Methods: We studied 48 naïve patients with chronic hepatitis C (25 males; median age 54 years; 19/48 with fibrosis absent/mild, S0–1, and 12/48 with advanced fibrosis, S4–6), who underwent a liver biopsy before starting standard antiviral therapy with pegylated interferon-α (PEG-IFNα) plus ribavirin. A fragment was stored for mRNA quantification by realtime-PCR; mRNA expression of ABCA1 in the liver biopsy was related to that measured in pooled normal liver tissue and presented as relative ratio (RR). ABCA1mRNA-RR was studied in relationship to HCV viral load, liver and metabolic tests, Ishak staging, IL28B rs12979860 and rs8099917 genotypes, response to treatment.
Results: There was no linear association between ABCA1 expression and viral load. Moreover, an inverse correlation existed between ABCA1-RR and the serum levels of aspartate aminotransferase (AST) (correlation coefficient −0.3, $p = 0.02$) and γ-glutamyl transferase (GGT) (correlation coefficient −0.3, $p = 0.01$): the higher were the AST and GGT levels, the lower was the expression of ABCA1. ABCA1 expression was significantly reduced in patients with

advanced fibrosis versus fibrosis absent/mild ($p < 0.05$), although plasma albumin levels had no relationship with ABCA1 expression. Moreover, the more advanced Ishak stages were, the higher were serum GGT levels ($p = 0.009$). ABCA1 expression was not related to gender, viral genotype, IL28B polymorphisms and rapid or sustained virological response to PEG-IFN plus ribavirin.

Conclusions: In chronic hepatitis C, ABCA1 expression in the liver does not have a linear relationship with viral load, but is reduced in association with hepatic inflammation, necrosis and advanced fibrosis. Since its alleged anti-tumoral properties, ABCA1 reduced expression in advanced stages of hepatitis C-related liver disease may be worth exploring as a contributor to progression towards hepatocellular carcinoma.

P0707

DIFFERENCES IN THE INTESTINAL MICROBIOTA IN PATIENTS WITH CHRONIC HEPATITIS C

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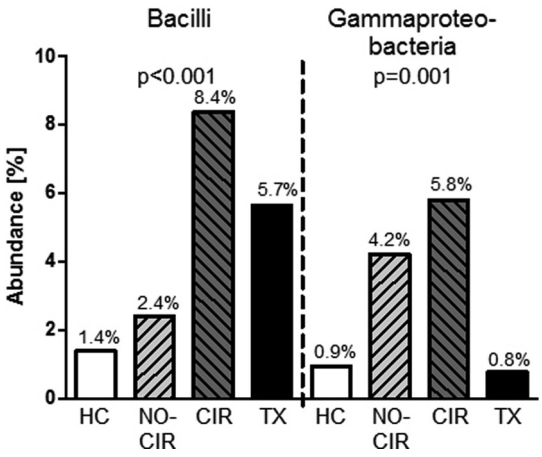
Background and Aims: The importance of the intestinal microbiota in the onset and clinical course of many diseases, including liver diseases like AFLD or NAFLD and liver cirrhosis, is becoming obvious. However, the role of the intestinal microbiota in chronic HCV infections remains unclear

Methods: We performed a cross sectional study comprising 51 patients infected with HCV [n = 18 w/o cirrhosis (NO-CIR); n = 28 with cirrhosis (CIR); n = 5 after solid organ transplantation (TX)] and 26 healthy controls (HC). Stool samples were prospectively collected, mixed with RNAlater and stored at −20°C. DNA was extracted using TRIzol reagent. The V1–2 region of the 16S rRNA gene was amplified followed by sequencing on the Illumina MiSeq platform. Sequences were quality filtered, trimmed collapsed and clustered and the annotation was performed using the RDP classifier. Statistical analysis was performed using SPSS 22 and Primer 6.

Results: A total of 4,328,784 reads were obtained out of which 3,060,948 (71%) reads passed the quality control giving a median of 35,132±16,611 per sample. The mean Shannon diversity index (H′) was significantly lower in individuals infected with HCV compared to HC (4.22±0.38 vs. 3.97±0.51; $p = 0.025$). In addition, H′ was significantly decreasing in HC, NO-CIR and CIR (4.22±0.38 vs. 4.10±0.51 vs. 3.86±0.53; $p = 0.037$). H′ showed a negative correlation with disease status (HC vs. NO-CIR vs. CIR) and the Child–Pugh score (CPS) ($r = -0.322$; $p = 0.006$ and $r = -0.478$; $p < 0.001$). Significant different community structures could be observed for the different stages of disease (HC vs. CO-CIR vs. CIR vs. TX). Bacilli and Gammaproteobacteria became more abundant during the course of HCV infection and descreased after organ transplantation ($p < 0.001$ and $p = 0.001$; Figure). Concomitantly, Alphaproteobacteria declined during the course of HCV infection and increased after TX ($p = 0.008$). Bacilli and Gammaproteobacteria showed a negative correlation with H′ ($r = -0.490$; $p < 0.001$ and $r = -0.412$; $p < 0.001$). In contrast, the frequency of Bacilli showed a positive correlation with CPS ($r = 0.505$; $p < 0.001$).

Conclusions: Our cross sectional study reveals significant differences between the microbial communities in patients during different stages of chronic HCV infection. The diversity is decreasing and the frequency of Bacilli and Gammaproteobacteria is increasing. These factors could be used to identify patients at risk of disease progression. However, these results need to be confirmed in long term studies



P0708

S-ADENOSYLMETHIONINE (SAM) ENHANCES ANTIOXIDANT ENZYME SYSTEMS, GLUTATHIONE BIOSYNTHESIS AND SWITCHES MAT2/MAT1 TURNOVER IN HEPATITIS C VIRUS EXPRESSING CELLS

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Background and Aims: SAM decreases HCV viral expression by unknown mechanisms. SAM is the main precursor of glutathione synthesis. Methionine adenosyltransferase 1A (MAT1A) enzyme is responsible of its biosynthesis in normal liver, but is replaced by MAT2 in liver regeneration and HCC. Our aim was to elucidate the mechanism(s) by which SAM decreases HCV expression, using a hepatoma cell line expressing HCV non-structural proteins.

Methods: Huh7 HCV-replicon and parental cells were treated with 1mM SAM. Total glutathione level was evaluated at different times by Ellman's recycling method (0–24 h). ROS level was determined by diclorofluorescein method (0–48 h), PDTC treatment was used as an antioxidant agent and hydrogen peroxide as a positive damage agent. Total RNA and protein were extracted (24–72 h). cDNA was synthesized and real time-PCR was performed to quantify HCV-RNA, SOD1, SOD2, catalase, thioredoxin 1, MAT1A and MAT2A expression and RPS18 as endogenous gene. Cellular and viral protein expression was evaluated by western blot using antibodies vs. HCV-NS3, HCV-NS5A, SOD1, SOD2, catalase, thioredoxin-1, MAT1A, MAT2A and actin.

Results: SAM treatment decreased HCV-RNA levels 50–70% compared to untreated control (24–72 h). Total glutathione levels increased upon 6 and 12 h post-treatment in replicon cells, but it was earlier in parental cells (2 h post-treatment). Transcriptional antioxidant protein expression (SOD1, SOD2 and thioredoxin1) was increased at different times but this effect was not observed for catalase. Interestingly, there was no significant change in ROS levels in both cells types upon SAM treatment, contrary to what was observed with PDTC, where an average of 30% reduction was detected. Finally, MATIA expression was increased (2.5 fold-times at 48 h) and MATII was decreased (from 24 h) upon SAM exposition at both transcriptional and translational level in both cell lines.

Conclusions: A posible mechanism(s) by which SAM decreases HCV expression could involve modulating antioxidant enzymes systems,

biosynthesis of glutathione and switching MAT2/MAT1 turnover in Hepatitis C virus expressing cells.

Viral hepatitis: Hepatitis C – b. Clinical (except therapy)

P0709

SURVIVAL OF PATIENTS INFECTED BY CHRONIC HEPATITIS C AND F0F1 FIBROSIS AT BASELINE AFTER A 15 YEARS FOLLOW-UP

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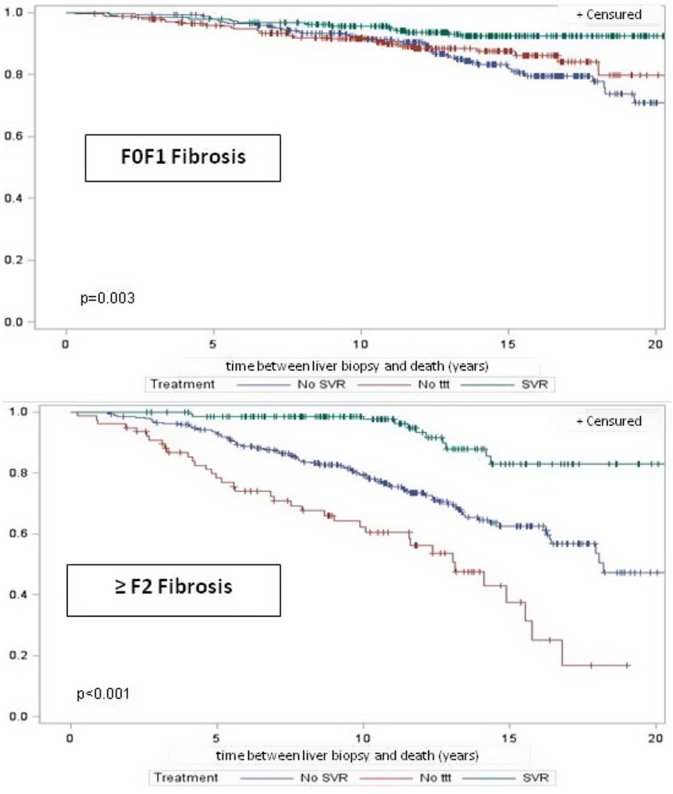
Background and Aims: The cost of direct antivirals for the treatment of chronic viral hepatitis C (CHC) is currently very high. In France, antiviral agents are restricted to patients with significant fibrosis (≥F2), and patients with F0F1 fibrosis are excluded. However long-term follow-up and survival of these patients without significant fibrosis is not well described.
Methods: Patients were selected from a single-center cohort of 4293 consecutive CHC patients included since 1992. Inclusion criteria were HCV-RNA positivity and liver fibrosis evaluated by biopsy at baseline. Survival was obtained from death certificate. Two groups were compared according to baseline fibrosis: F0F1 versus ≥F2.



Results: 1381 patients were included (60% male, 52.4% G1 and 19.4% G3, 38.8% drug users). At baseline median age was 43 years [36–53], stage of fibrosis: F0F1 59.4%, F2 18.5%, F3 9.5% and F4 12.6%. Compared to F2, F0F1 patients were younger (42±12 vs 50±13 years, $p < 10^{-3}$), had lower prevalence of male (57% vs 64.5%, $p = 0.005$) and antiviral treatment (68.8% vs 86.1%, $p < 10^{-3}$) and a higher prevalence of sustained virological response (SVR: 48% vs 34.9%, $p < 10^{-3}$). After a median follow-up of 4.6 [2.9–7.7] years, a 2nd liver biopsy was performed in 157 F0F1 patients (19.1%), 50 F2 patients (19.5%) and 28 F3 patients (21.4%). An increased fibrosis stage to F3 or F4 was observed in 15.3% of F0F1 patients, 48% of F2 patients and 46.7% in F3 patients (to F4). After a median follow-up of 11.9 years [8.7–15.5], 248 patients died (16.7%), more frequently in ≥F2 patients (24.4%) than in F0F1 patients (11.5%), $p < 10^{-3}$. Median survival at 5, 10 and 15 years after the first liver biopsy was 97.4%, 93.1% and 87% in F0F1 patients and 93.2%, 83.4% and 65.4% in ≥F2 patients. Survival was better in treated patient with SVR than in untreated patient or treated without SVR regardless fibrosis stage, as shown in the figure.
Conclusions: Antiviral treatment for F0F1 patients at baseline should be considered due to (i) an evolution to severe fibrosis in at least 15%, (ii) a better response to treatment (SVR achieved in 48%), (iii) an improved survival in patients with SVR compared to those without SVR demonstrated in both F0F1 and F≥2 groups.

P0710

ROLE OF HEPATITIS C VIRUS (HCV) IN THE EARLY ARTERIOSCLEROTIC PROCESS AND AUTONOMIC DISFUNCTION

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Background and Aims: HCV infection may not only affects the hepatocytes, provoking chronic hepatitis, cirrhosis and HCC, but it also involves nerves, lymphocytes and endothelial cells determing an *extrahepatic disease*. Therefore we investigated the role of HCV in the atherosclerosis, autonomic dysfunction and endothelial injury.
Methods: From January 2011–2012, we recruited 101 subjects (48M/53F; aged 50±10 yrs) with chronic hepatitis C, who were matched for age, sex, BMI and cardiovascular risk factors to a control group without HCV. Eighty-four cases (83%) were treated with Peg-IFN+RBV, and 48 cases (56%) achieved the sustained viral eradication long-lasting, while 53 cases remained HCV-RNA positive. All cases underwent measuring of: (a) intima-media thickness (IMT) with US-Doppler of carotid arteries (CC, common carotid; B, bulb carotid, ICA, internal carotid), (b) flow-mediated dilation (FMD) of brachial artery to evaluate endothelial function, (c) Tilt, Valsalva, Hand-grip and Deep tests, to analyze peripheral autonomic nervous system and (d) PAI-1 plasma levels to define the involvement in trombotic and artheriosclerotic risk.
Results: The IMT for *CC, B* and *ICA* was significantly higher in patients with HCV compared to controls (1.1±0.3; 1.3±0.5; 1.1±0.4 vs 0.9±0.2; 0.9±0.3; 0.9±0.2, respectively; all $p < 0.01$), while FMD was significantly lower (12.8±6.4 vs 21.8±9.7; $p < 0.01$). Using Pearson correlation, the relationship between IMT and FMD in cases with and without HCV showed a significant difference only in controls (*CC,* $r = -0.48$; *B,* $r = -0.5$; *ICA,* $r = -0.53$; all $p < 0.01$), but not in cases with HCV (p = ns). Results obtained by autonomic tests significantly differed between subjects with and without HCV (Tilt, 1.1±0.2 vs 1.3±0.1; Valsalva, 1.3±0.2 vs 1.5±0.2; Hand, 2.0±1.7 vs 6.5±3.7; Deep, 1.2±0.2 vs 1.4±0.1; all $p < 0.01$), but between subgroups of cases with or without HCV-RNA, there were no statistical differences. Also PAI-1 levels did not result statistically different either between subjects with and without HCV (6.6±4.7 vs 6.0±4.8; p = ns) or cases with and without HCV-RNA (5.9±4.8 vs 7.4±4.4, p = ns).
Conclusions: HCV appears to play a pathogenetic role in early atherosclerotic process and autonomic dysfunction, thus, it might be likely that during the course of chronic hepatitis C irreversible damages are induced especially into the endothelium and the nerv us autonomic system. This events might recommend a

cardiovascular and neurologic follow-up in patients with present and past HCV infection.

P0711

GENOME-WIDE ASSOCIATION STUDY OF RESPONSE TO SOFOSBUVIR–RIBAVIRIN TREATMENT IN GT2/3 HCV PATIENTS

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Background and Aims: We examined the association of host genetic variants with treatment outcome in patients participating in studies of Sofosbuvir (Sovaldi™, SOF)+RBV.
Methods: 572 patients infected with HCV genotype 2 or 3 who received 12 or 16 weeks of SOF+RBV therapy in Phase 2 and 3 studies (ELECTRON, FISION, POSITRON, FUSION) were genotyped on the Omni5Exome chips. 431 samples from Caucasian patients infected with HCV (GT2=167; GT3=264) passed quality control and were retained as the final discovery dataset, containing 130 cases (relapse) and 301 controls (SVR). A logistic regression model was fitted to the data, adjusting for principal components and clinical characteristics. The candidate SNPs identified in the initial discovery set were genotyped in patients from 3 independent clinical trials (VALENCE, n = 280 patients infected with GT2 or GT3 HCV; PHOTON-1, n = 109 patients co-infected with HIV and HCV GT1, 2 or 3; NEUTRINO, n = 130 patients infected with GT1, 4, 5 or 6 HCV) for validation.

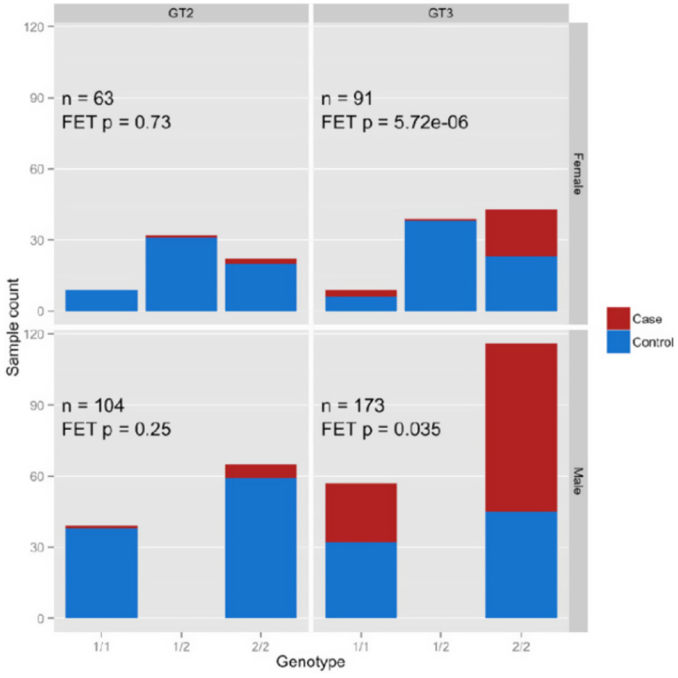

Figure 1. Gender and HCV genotype stratified effect of rs787078 on SVR and relapse.

Results: The top SNP identified in the discovery set (rs787078, $p = 7.28 \times 10^{-8}$) mapped to the downstream region of the gene *DDX3X*, located on the X chromosome with a MAF=0.35. *DDX3X* may be involved in regulation of viral replication, including HCV assembly. After Bonferroni multiple testing corrections, this SNP did not reach genome-wide significance ($p = 1.52 \times 10^{-8}$) but it did deviate from neutrality. When patients were stratified by HCV genotype and gender, the rs787078 association was primarily driven by female patients infected by genotype 3 HCV (Figure 1). Within the GT3 subset, SVR rates were 56% in males and 92% in females with the protective allele, and patients homozygous for the major allele had a greater relapse risk regardless of gender. However, data from the three independent, separate HCV studies failed to replicate the observed association.
Conclusions: The current study is the first comprehensive genome-wide investigation of the role of human genetic variants associated with SVR following HCV treatment with SOF-based all-oral regimens. No significant genetic associations were observed linking SVR among Caucasians. The *DDX3X* gene region was identified as a candidate with biological relevance. However, this association was not confirmed by data from independent cohorts. This study emphasizes the need for rigorous validation of hypothesis-generating genetic findings. It remains unclear whether this variant or *DDX3X* has a functional role in SVR outcome in HCV patients.

P0712

THE PHARMACOKINETICS OF GS-5816, A PANGENOTYPIC HCV-SPECIFIC NS5A INHIBITOR, IN HCV-UNINFECTED SUBJECTS WITH SEVERE RENAL IMPAIRMENT

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Background and Aims: GS-5816, a potent pangenotypic HCV NS5A inhibitor, is in clinical development for the treatment of genotype 1–6 chronic HCV infection in combination with sofosbuvir (SOF). GS-5816 is primarily eliminated in the feces as parent drug and metabolites with <1% of the dose excreted in the urine. This study evaluated the short-term safety and pharmacokinetics (PK) of GS-5816 in subjects with severe renal impairment (RI) versus matched control subjects with normal renal function (NF). Results from this study will inform dosing recommendations for GS-5816 and, in part, SOF/GS-5816 in this population.
Methods: Ten subjects with stable severe RI (CL_{cr} <30 mL/min), and corresponding healthy subjects with NF (CL_{cr} ≥90 mL/min), matched for age (±10 yrs), sex, and BMI (±15%), received a single dose of GS-5816 100 mg after a light meal (~400 kcal, 30% fat) followed by intensive PK sampling over 120 hours. Safety assessments were performed throughout the study. Comparative statistics for GS-5816 AUC and C_{max} were calculated with an exposure increase >100% being considered clinically relevant.
Results: GS-5816 was generally safe and well tolerated in all study subjects. All treatment-emergent AEs were Grade 1 (mild) in severity. No AEs occurred in greater than 1 subject. An approximately 50% increase in GS-5816 AUC was observed in subjects with severe RI compared to subjects with NF. Maximum GS-5816 concentrations (C_{max}) were similar in both groups. Plasma protein binding was similar in both groups. The modest increase in GS-5816 exposure is consistent with changes in drug transport and/or (hepatic) metabolism associated with renal impairment.
Conclusions: GS-5816 exposure (AUC and C_{max}) was not substantially altered, and no change in protein binding was

observed in subjects with severe RI relative to those with NF. GS-5816 may be administered without dose adjustment to patients with mild, moderate, or severe renal impairment.

GS-5816	Mean (%CV)		GMR% (90% CI)
PK Parameter	Severe RI (N = 10)	Normal function (N = 9)	Severe RI:NF
AUC_{inf} (ng·h/mL)	8110 (32.4)	5650 (31.2)	150 (117, 192)
AUC_{last} (ng·h/mL)	7970 (31.8)	5600 (31.2)	149 (117, 190)
C_{max} (ng/mL)	732 (24.1)	703 (28.1)	111 (90.8, 135)

P0713

THE IMPACT OF LEDIPASVIR (LDV)/SOFOSBUVIR (SOF) COMBINATION ON HEALTH-RELATED QUALITY OF LIFE (HRQL) AND PATIENT-REPORTED OUTCOMES (PROS) IN CIRRHOTIC PATIENTS WITH CHRONIC HEPATITIS C (CH-C): THE SIRIUS STUDY

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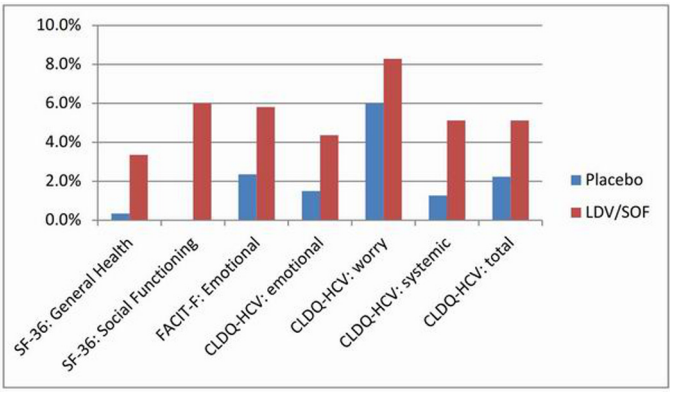
Background and Aims: Interferon (IFN) and ribavirin (RBV) can negatively impact PROs. In contrast, IFN-RBV-free regimens may improve PROs during treatment. The aim is to compare PROs during treatment with LDV/SOF to placebo and to LDV/SOF+RBV.
Methods: Treatment-experienced CH-C genotype 1 patients with compensated cirrhosis (N = 154) were randomized to be treated with 24 weeks of LDV/SOF or with 12 weeks of placebo followed by 12 weeks of LDV/SOF+RBV (SIRIUS clinical trial). While blinded to their HCV RNA results, patients completed 4 validated PRO questionnaires [Short Form-36 (SF-36), Chronic Liver Disease Questionnaire-HCV (CLDQ-HCV), Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), and Work Productivity and Activity Index: Specific Health Problem (WPAI:SHP)] which were administered at baseline, during and post-treatment.
Results: The study cohort was 74% male, age 56.5±9.2 years, and 96.75% achieved SVR. Baseline PRO scores were similar between the study arms. As early as 4 weeks after treatment, patients receiving LDV/SOF showed improvement of mental component summary score (MCS) of SF-36, overall CLDQ-HCV score, emotional well-being score of FACIT-F and activity score of WPAI:SHP (all p-values <0.05), while no significant changes in these scores were noted in the placebo group. After 12 weeks of treatment with LDV/SOF, improvements in social functioning and general health of SF-36 were also noted ($p < 0.05$) (Figure 1). In the second 12 weeks of the study, patients receiving LDV/SOF continued to show gains in PROs (up to +9.2% on a 0–100% scale from baseline, $p < 0.05$), while patients receiving LDV/SOF+RBV had less PRO gains or no improvement. Nevertheless, regardless of the regimen, achieving SVR-12 was associated with significant improvement in all aspects of PROs (up to +12.2% in both regimens). Multivariate analyses showed that, in addition to previously reported factors (pretreatment depression, anxiety, insomnia, older age, diabetes, all p-values <0.05), receiving RBV was independently associated with PRO impairment (beta up to −10.5%, $p < 0.05$).
Conclusions: Treatment-experienced cirrhotic patients experience a notable improvement of their PROs with LDV/SOF. Achieving SVR-12 is associated with improvement in most of the PROs in this difficult to treat group of patients with CH-C.



P0714

IMPACT OF PRIORITIZING TREATMENT IN A HIGH RESOURCE SETTING – MINIMIZING THE BURDEN OF HCV RELATED DISEASE IN 15 YEARS

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Background and Aims: Even in a high resource setting, prioritizing direct acting antiviral (DAA) treatment for Chronic Hepatitis C (CHC) is an issue. The effect of DAA treatment on the burden of disease can be measured on many parameters including preventing progression to advanced liver disease and halting transmission to reduce prevalence. In Denmark, the majority of CHC patients have been infected through needle sharing and intravenous drug use. Prevalence is comparatively low, approximately 0.4% in the +15 year old population (21,000 patients), and most patients are between 45–55 years of age. This relatively young infected population is beginning to progress to advanced disease, with cirrhosis identified in ~20% of patients followed for CHC. Treatment with interferon free DAA regimens is now available for patients with biopsy or FibroScan proven severe fibrosis (F3) or cirrhosis, however little is known about the impact of these new criteria.
The aim of this study is to evaluate
1. The impact of current treatment strategies on the number of viremic patients, liver related mortality, decompensated cirrhosis and HCC
2. The numbers needed to treat to halve prevalence by 2030

Methods: The model for CHC disease progression developed by Razavi et al was applied and fitted to the Danish setting. **Base case scenario (pre 2014):** interferon based therapy and annual treatment of 100 ≥F2 patients. **DAA era (2014):** increased treatment efficacy, eligibility and numbers (n = 200); restricted to patients with fibrosis ≥F3. **After 2015:** Scenarios examining the effect of expanding treatment (less restriction on degree of fibrosis) and increased treatment were added.
Results: Treating 400 or 600 patients with ≥F3 fibrosis from 2014 onward would reduce liver related mortality by 50% or 75%, respectively, HCC by 50% and 75%, and decompensated cirrhosis by 55% and 85% by 2030. Total viremics would be reduced by 30%. To

reduce prevalence by half by 2030, 880 patients have to be treated annually if treatment is restricted to fibrosis ≥F2 starting in 2016. Due to the undiagnosed population, annual treatment numbers of ≥F3 patients cannot be increased beyond about 700 after 4–5 year of treatment

Conclusions: If the aim is to reduce liver related mortality, HCC and decompensated cirrhosis, the strategy of prioritizing treatment for patients with ≥F3 is effective but does little by way of reducing prevalence. Finding undiagnosed patients with severe fibrosis and cirrhosis is essential to further reduce liver related morbidity and mortality.

P0715

STEADY-STATE PHARMACOKINETICS AND SAFETY OF COADMINISTRATION OF PAN-GENOTYPIC, DIRECT ACTING PROTEASE INHIBITOR, ABT-493 WITH PAN-GENOTYPIC NS5A INHIBITOR, ABT-530, IN HEALTHY ADULT SUBJECTS

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Background and Aims: A next generation direct acting antiviral (DAA) combination of ABT-493 (NS3/4A protease inhibitor discovered by AbbVie and Enanta) + ABT-530 (NS5A inhibitor) is being developed for the treatment of chronic hepatitis C (HCV) genotype 1–6 infection. Each compound demonstrated potent antiviral activity following 3-day monotherapy (AASLD 2014). The purpose of this study was to evaluate pharmacokinetics (PK) and safety of several different dose levels of ABT-493 and ABT-530 when given in combination.

Methods: Open-label 5-arm, cohort study in 72 healthy subjects with assessment of steady-state PK and safety of ABT-493 (100, 400, 700 or 1200 mg QD) combined with ABT-530 (40, 120, 160 or 200 mg QD). Intensive blood sampling for determination of ABT-493 and ABT-530 concentrations was performed. Safety and tolerability were assessed throughout the study.

ABT-530 dose	ABT-493 dose	Results
40 mg	100 mg	ABT-530 had minimal impact on ABT-493 ABT-493 increased ABT-530 to 1.5×
40 mg	400 mg	ABT-530 had minimal impact on ABT-493 ABT-493 increased ABT-530 to 6×
120 mg	400 mg	ABT-530 slightly increased ABT-493 exposures ABT-493 increased ABT-530 to 3–4×
160 mg	700 mg	ABT-530 slightly increased ABT-493 exposures ABT-493 increased ABT-530 to 5–7×
200 mg	1200 mg	Arm was prematurely discontinued, no steady state data were available

Results: ABT-493 exposures increased in a greater than dose proportional manner across the 100 mg to 1200 mg dose range. In the presence of ABT-530 (120 and 160 mg), geometric mean ABT-493 exposures were higher (41% to 83%) compared with ABT-493 administered alone. ABT-530 exposures increased in a greater than dose-proportional manner across the 40 mg to 120 mg dose range and approximately dose-proportionally from 120 mg to 200 mg. Co-administration with 400 mg ABT-493 increased ABT-530 120 mg exposures to 3- to 4-fold and 40 mg ABT-530 exposures to 6-fold compared with ABT-530 administered alone (Table). In contrast, 100 mg ABT-493 increased 40 mg ABT-530 exposures to 1.5-fold (Table). The ABT-493 1200 mg + ABT-530 200 mg arm was prematurely discontinued due to ABT-493 exposures exceeding the pre-specified upper exposure limit by the protocol. Two subjects discontinued prematurely, one was due to a Grade 2 adverse event of allergic dermatitis after a single dose of ABT-493, and the other was due to an asymptomatic Grade 3 ALT elevation after 7 days of 1200 mg ABT-493. One subject developed asymptomatic, isolated, Grade 3 total bilirubin elevation. Described laboratory abnormalities were observed with 1200 mg or 700 mg ABT-493, and improved/normalized after conclusion of dosing.

Conclusions: ABT-530 at doses of 120 or 160 mg QD, but not 40 mg QD, slightly increased ABT-493 exposures (≤83%). ABT-493 dose-dependently increased the exposures of ABT-530. Both ABT-530 and ABT-493 were well tolerated and not associated with clinically significant/relevant laboratory abnormalities when the ABT-493 dose was less than 700 mg QD.

P0716

THE SIGNIFICANCE OF PLATELET MICROPARTICLES IN PATIENTS WITH CHRONIC HEPATITIS C AND THEIR ASSOCIATION WITH ANTIVIRAL TREATMENT AND SMOKING

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Background and Aims: Platelet-microparticles (PMPs) are platelet-derived membrane vesicles generated by cell activation and apoptosis which have been involved in cardiovascular diseases and atherosclerosis. Chronic hepatitis C (CHC) is associated with increased atherosclerosis, but the effect of antiviral treatment on the atherogenic potential of CHC has not been adequately studied so far. The aim of this study was to evaluate PMPs levels before and after antiviral treatment in CHC patients.

Methods: 28 CHC patients (13 G1/G4, 15 G3) were included, whereas 20 healthy volunteers (HV) and 20 patients with non-alcoholic fatty liver disease (NAFLD) were used as controls. Patients with cardiovascular diseases, diabetes, anticoagulant and antiplatelet treatment were excluded. All CHC patients were treated with pegylated-interferon/ribavirin. Twenty-four (86%) achieved sustained virological response (SVR). PMPs levels were determined by flow-cytometry (CD61-Annexin V) at baseline in all CHC patients and controls as well as at end of treatment (EOT) and 24 weeks post-treatment (SVR24) in all CHC patients.

Results: PMPs levels at baseline were higher in CHC compared to NAFLD patients ($P < 0.001$) and HV ($P = 0.007$). Higher PMPs levels at baseline were observed in smokers ($n = 18$) compared to non smokers ($n = 10$) with CHC ($P = 0.006$). Among smokers from all groups, CHC patients had higher PMPs at baseline compared to NAFLD ($P = 0.001$) and HV ($P = 0.024$). During antiviral treatment in all CHC patients, PMPs levels declined from baseline to both EOT ($P = 0.035$) and SVR24 ($P = 0.006$). PMPs levels decline during treatment was mainly observed in smokers [PMPs declined significantly from baseline to EOT ($P = 0.004$) and SVR24 ($P = 0.009$)]. Only patients achieving SVR had a significant decline in PMPs from baseline to SVR24 ($P = 0.018$), while those without SVR did not experience any significant change. Patients with G1/G4 experienced a higher decline in PMPs compared to G3 patients. PMPs levels at EOT and SVR24 in all CHC patients as well as in smokers became similar to those in control groups.

Conclusions: The higher PMPs levels in CHC patients and particularly in smokers further support the atherosclerotic potential of CHC and suggest a potentially synergistic effect of smoking and CHC on the atherosclerotic process. Since PMPs levels in CHC patients with SVR do not differ from those in controls, the atherosclerotic potential of CHC seems to be abolished by effective antiviral treatment.

P0717

THE PRESENCE OF A PNPLA3 (RS738409) SINGLE NUCLEOTIDE POLYMORPHISM (SNP) IS NOT ASSOCIATED WITH INCREASED RISK FOR FIBROSIS, PORTAL HYPERTENSION, AND HEPATIC STEATOSIS IN HIV/HCV-COINFECTED PATIENTS

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Background and Aims: HIV/HCV-coinfected patients often show hepatic steatosis, faster fibrosis progression, and a higher risk of developing end-stage liver disease. While several risk factors for faster fibrosis progression have been established, the role of a genetic variation of the patatin-like phospholipase domain-containing protein 3 (PNPLA3) remains unclear. Thus, we examined the impact of the PNPLA3 single nucleotide polymorphism (SNP rs738409) on liver steatosis and fibrosis in a cohort of HIV/HCV-coinfected patients.
Methods: 177 HIV/HCV-coinfected patients with available data on PNPLA3 (SNP rs738409) and IL28B (SNP rs12979860) were enrolled. Liver fibrosis and steatosis was staged either by liver biopsy or by transient elastography [FibroScan® using the Controlled Attenuation Parameter (CAP™) module]. Parameters associated with advanced liver fibrosis (≥F3) and portal hypertension (hepatic venous pressure gradient, HVPG) were examined. Fibrosis progression rate (FPR in METAVIR F stages/year) was calculated using the estimated year of HCV infection. Sustained virological response (SVR) rates were assessed across PNPLA3 genotypes.
Results: Patients tested positive for a PNPLA3 minor (G/C, n = 66) or major (G/G n = 9) risk allele (42.4%) showed comparable fibrosis stages (both median F2; p = 0.106) and a similar amount of hepatic steatosis (CAP: 200.3±44.7 vs. 217.7±56.2; p = 0.484). Advanced liver fibrosis was neither associated with PNPLA3 (p = 0.418) nor with IL28B-genotype (p = 0.637) but was associated with HCV-GT 3 (p < 0.001), lower CD4 count (p = 0.024), lower CD4 nadir (p = 0.011) and higher AST values (p = 0.016). Fibrosis progression rate (0.21±0.27 vs. 0.46±0.74 units/year; p = 0.313) and HVPG-values (3.7±2.5 vs. 4.6±3.1 mmHg; p = 0.172) were comparable in patients with and without PNPLA3 risk alleles.
SVR rates to PEGIFN/RBV therapy were 68.5% in PNPLA3-C/C, 51.6% for G/C, and 100% for G/G. However, the proportion of IL28B-C/C genotype in the PNPLA3 risk allele subgroup was surprisingly high with 54.7%.
Conclusions: The presence of a PNPLA3 risk allele had no influence on liver fibrosis progression, portal hypertension, or hepatic steatosis in HIV/HCV-coinfected patients. The small number of patients with a major PNPLA3 risk allele may represent a potential limitation. However, our data indicated that the presence of a PNPLA3 risk-allele does not comprise virological response rates to IFN-based therapies in IL28B-C/C HIV/HCV-coinfected patients.

P0718

COST-EFFECTIVENESS OF INTERFERON-FREE THERAPY FOR HEPATITIS C IN GERMANY – AN APPLICATION OF THE EFFICIENCY FRONTIER APPROACH

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Background and Aims: The approval of direct-acting antivirals for Interferon-free treatment revolutionized the therapy of chronic Hepatitis C infection. As of August 2014, two treatment regimens for genotype 1 infection received approval in the European Union: Sofosbuvir and Ribavirin for 24 weeks and Sofosbuvir and Simeprevir with or without Ribavirin for 12 weeks. We aim to analyze the cost-effectiveness of both regimens in Germany.
Methods: We set up a Markov model with a lifetime horizon to simulate immediate treatment success and long-term disease progression for treatment-naive patients. The model analyzes both shortterm and longterm costs and benefits from the perspective of the German Statutory Health Insurance. We apply the efficiency frontier method, which was suggested by German Institute for Quality and Efficiency in Health Care for cost-effectiveness analysis in Germany.
Results: The efficiency frontier is defined by dual therapy and first generation direct-acting antiviral Boceprevir, yielding a maximum of € 1,447.69 per additional percentage point of sustained virologic response gained. Even without rebates, Sofosbuvir/Simeprevir is very close with € 1,560.13 per additional percentage point. It is both more effective and less expensive than Sofosbuvir/Ribavirin.
Conclusions: In addition to higher sustained virologic response rates, new direct-acting antivirals save long-term costs by preventing complications such as liver cirrhosis, hepatocellular carcinoma and ultimately liver transplants, thereby offsetting part of higher drug costs. Our findings are in line with the guidance published by German Society for Gastroenterology, Digestive and Metabolic Diseases, which recommends Sofosbuvir/Simeprevir for Interferon ineligible or intolerant patients.

P0719

HEALTHCARE COSTS BY STAGE OF LIVER DISEASE IN CHRONIC HEPATITIS C PATIENTS IN THE UNITED STATES

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Background and Aims: Hepatitis C virus (HCV) is a leading cause of chronic liver disease worldwide and can lead to significant increases in healthcare resources. The aim of this study was to examine all-cause direct medical and pharmacy costs in US HCV patients in various stages of liver disease compared to patients without HCV.
Methods: This US-based cohort study was conducted comparing all-cause healthcare (medical and pharmacy) costs of adult patients with or without HCV using MarketScan's Commercial insurance claims database for the period 5/2000 through 9/2013. HCV patient cohorts were defined by their most severe liver disease stage based on ICD-9 diagnosis codes, least to most severe stage: HCV non-cirrhotic (NCHCV), compensated cirrhotic (CCHCV), decompensated cirrhotic (DCCHCV), hepatocellular carcinoma (HCCHCV), and liver transplant (LTHCV). The HCV patient index date was defined by their most severe liver disease diagnosis date. Five non-HCV patients were randomly selected for each HCV patient. Non-HCV patient index date was randomly chosen during the study period. Patients were required to have at least one-year of continuous enrollment pre and post index date. Patients were excluded if <18 years old, or had a diagnosis for HIV or Hepatitis B. Propensity score-adjusted bin bootstrapping (PSB) method was used to estimate the difference in total per patient per month (PPPM) healthcare costs between each severity stage cohort and the non-HCV cohort. The PSB model accounts for the skewed nature of cost data. Age, gender, Charlson comorbidity score and previous HCV treatment experience were covariates in the PSB model. Costs are in 2013 US dollars.
Results: All HCV liver disease severity cohorts were statistically significantly (p < 0.05) older and male with higher mean Charlson Comorbidity Index scores than the non-HCV cohort. The total PPPM unadjusted all-cause mean costs were statistically significantly higher for all HCV cohorts compared to the non-HCV cohort (Table). The PPPM adjusted all-cause mean cost (95% CI) difference between

Table (abstract P0719): Cost by Liver disease stage

Parameter	Non-cirrhotic HCV	Compensated cirrhosis HCV	Decompensated cirrhosis HCV	Hepatocellular carcinoma HCV	Liver transplant HCV	No chronic HCV
Number of patients	20,873	5216	11,135	1802	1434	199,824
PPPM Unadjusted all-cause costs (95% CI)	$2139 ($2077–$2201)	$2491 ($2361–$2622)	$5038 ($4799–$5277)	$10,798 ($10,145–$11,451)	$30,100 ($27,308–$32,893)	$434 ($426–$443)
PPPM Adjusted all-cause cost difference between disease severity stage and no chronic HCV (95% CI)	$1633 ($1572–$1697)	$1885 ($1778–$1999)	$3360 ($3172–$3561)	$7904 ($7250–$8581)	$21,589 ($18,894–$23,457)	–

each severity stage and the non-HCV cohort were statistically significant ranging from a difference of $1633 PPPM in the NCHCV cohort up to a difference of $21,589 PPPM in the LTHCV cohort (Table).

Conclusions: Mean healthcare costs increased as liver disease severity stage worsened. These costs were significantly higher in all the HCV liver disease stage cohorts compared to a cohort without HCV.

P0720

IMPROVEMENT OF ADVANCED LIVER FIBROSIS AND REGRESSION OF LIVER CIRRHOSIS IN PATIENTS WITH HEPATITIS C GENOTYPE 1 AFTER HCV PROTEASE INHIBITOR-BASED TRIPLE THERAPY

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Background and Aims: Triple therapy with pegylated interferon alpha, ribavirin and a HCV protease inhibitor (PI) is currently the standard of care for chronic hepatitis C genotype 1 (GT1) in many European countries. Data on the course of liver fibrosis and cirrhosis regression after treatment with PI are scarce. The same applies to the influence of sustained virologic response (SVR) or other factors in the evolution of liver fibrosis. We aim to evaluate the impact of PI-based therapy on liver fibrosis measured by transient elastography in clinical practice.

Methods: A multicenter observational and prospective study was conducted. Patients with GT1, treated with PI-based therapy (telaprevir or boceprevir), with advanced liver fibrosis (>9.5 kPa) at baseline were included. Liver stiffness measurements were performed with the FibroScan®502 device and M probe at the baseline and 24 weeks after the end of treatment. Patients with baseline liver stiffness values above 12.5 kPa were considered to have cirrhosis. By now, 72 patients have completed treatment, following them for 24 weeks (age/male/RNA/subtype/ IL28B/ previous treatment/type of PI/fibrosis/SVR 12).

Results: Baseline patient characteristics: 58 male, median 52±8.9 years, prior treatment: 75%, subtype: 1a: 34.7%, 1b: 56.9%, IL28B CC: 18%, median baseline liver stiffness: 13.2±10.2 kPa (F4: 48.6%, no F4: 51.4%), 65.3% were treated with telaprevir and 34.7% with boceprevir. 49 of 72 (68.05%) patients achieved SVR. In patients who achieved SVR the median intra-patient decrease relative to baseline at the end of follow-up was −3.8 kPa, vs +1.1 kPa in the patients who did not achieve an SVR ($p = 0.001$). Liver stiffness decreased more than 30% in 52% of patients with SVR and in 21% of patients who did not achieve SVR ($p = 0.01$). Three patients in the non-SVR group and one in the SVR group progressed to cirrhosis. 14 out of 23 (60.87%) SVR patients with cirrhosis at baseline demonstrated a regression to values below 12.5 kPa after therapy.

Conclusions: In patients with advanced liver fibrosis at the beginning of PI-based therapy, liver stiffness is significantly reduced after treatment, but only if they achieved SVR, in a short term follow up of 24 weeks after the end of treatment. These findings even suggest the possibility of regression of liver cirrhosis after SVR in a significant proportion of patients.

P0721

VIRAL AND HOST PARAMETERS IN ASSOCIATION WITH OUTCOME OF ACUTE HEPATITIS C IN HIV-COINFECTED PATIENTS

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Background and Aims: The HCV (hepatitis C virus) NS3 protease is essential for HCV replication, is a target for direct acting antivirals and cleaves the host protein CARDIF, which is involved in innate immune signaling. In this study, we investigated the NS3 protease evolution and function as well as other viral and host parameters in correlation with the outcome of acute hepatitis C.

Methods: Blood was collected prospectively from 82 consecutive patients with HIV (human immunodeficiency virus) coinfection at diagnosis of acute hepatitis C and every 4 weeks thereafter. Liver enzymes, HCV viral load (VL) and genotype, IP10 levels (interferon-gamma inducible protein) and IL28B genotype (rs129797860, rs8099917) were investigated in correlation with outcome. Longitudinal clonal NS3 protease sequencing was performed for a quasispecies characterization (Hamming distance/Shannon entropy) and major patient NS3/4A variants were expressed in U2OS cells to analyse CARDIF cleavage.

Results: We observed a spontaneous clearance rate of 13% (n = 11). Treatment was initiated during the acute phase in 43 (52%) individuals, 24 (30%) patients developed a chronic HCV infection (positive HCV-RNA 6 months after diagnosis) and the outcome is unclear for 4 (5%) persons. In comparison to a control group with a long-term chronic hepatitis C, acutely infected individuals displayed a significantly higher NS3 quasispecies diversity and complexity (both $P < 0.001$). However, the mean Hamming distance/Shannon entropy at diagnosis was comparable in patients with spontaneous clearance versus treatment-induced SVR (sustained virologic response) (both 0.010/0.031) or development of chronic hepatitis C (0.008/0.025). Longitudinal NS3 quasispecies kinetics showed a significant trend to a decreasing quasispecies diversity and complexity (both $P < 0.05$) within 4 weeks after diagnosis in patients with spontaneous clearance compared to SVR or progression to chronic hepatitis C. Furthermore, spontaneous clearance is predictable by a significantly higher HCV VL decline compared to development of a chronic hepatitis C (3.5 versus 1.7 log10 IU/mL,

P = 0.003). CARDIF was cleaved similarly by individual patient NS3/4A proteases independent of the outcome.
Conclusions: We detected a rapid NS3 quasispecies evolution during acute hepatitis C. Together with a high viral load decline, an early decreasing NS3 quasispecies evolution is indicative for a spontaneous clearance while the natural course of acute hepatitis C correlated not with NS3 CARDIF cleavage activity.

P0722

NON-INVASIVE DETECTION, STRATIFICATION AND TREATMENT OF CHRONIC HEPATIC C RELATED LIVER DISEASE AMONGST SUBSTANCE USERS IN THE COMMUNITY: A PROSPECTIVE STUDY

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Background and Aims: Despite discovery of direct acting antiviral agents (DAA), a two–three fold increase in hepatitis C virus (HCV) detection and treatment is necessary to reduce the national HCV disease burden. Ninety percent of HCV positive individuals in England are people who inject drugs (PWID) with poor engagement with health services
Methods: Two-year prospective study (December 2013 to November 2015) conducted at a large substance misuse service in SE England. Individuals offered dry blood spot testing (DBST), mobile transient elastography (TE), and HCV treatment (pegylated interferon/ribavirin/telaprevir). Clinically significant hepatic fibrosis defined as liver stiffness measurement (LSM) ≥7.5 kPa.
Results: To date, 165 individuals have been recruited, mean age 39.8±8.9 yrs, 84.8% male. One hundred and forty-one (85.5%) had history of injecting drug use (IDU) with 59 (35.8%) currently injecting, 143 (86.7%) reported past alcohol use with 62 (37.6%) currently drinking. Eighty-three (50.3%) had had a psychiatric diagnosis. Uptake of DBST was 75 (45.5%), the main reason for declining being recent prior testing. Overall prevalence of positive serological markers/PCR were: HBcAb 28.8% (n = 42), HCV antibody 62.2% (n = 97) and HCV PCR 75.2% (73/97) (genotype 3a 42.8%, 1a 34.3%). Of those with a positive HCV PCR (n = 73), 62 (84.9%) underwent TE, mean LSM being 7.7±6.6 kPa, 21 (33.9%) having LSM ≥7.5 kPa including13 (21%) with cirrhosis (LSM ≥12 kPa), one of whom had decompensated disease. One of the 73 with a positive HCV PCR none had had prior treatment. Fifteen (20.5%) were not treatment candidates (chaotic life style), interferon was inappropriate and or contraindicated in 35 (47.9%) and only 23 (31.5%) eligible were for interferon based treatment. Thirteen have commenced therapy (nine genotype 3a, three 1a and one 2b), of whom 4 have successfully completed with 8 achieving rapid virological response
Conclusions: Both DBST and mobile TE have good uptake amongst PWID though prevalence of a positive HCV antibody remains high (62.2%). Despite a mean age of <40 yrs about a third of PWID have clinically significant HCV related hepatic fibrosis with 1:5 having established cirrhosis. Though community based interferon therapy is feasible in PWID despite ongoing drug and alcohol use, it is contraindicated in approximately 50% emphasizing the urgent need for interferon-free regimens in this vulnerable population

P0723

HCV REINFECTION INCIDENCE DECLINE AMONG HIV-POSITIVE MEN WHO HAVE SEX WITH MEN IN THE UK

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Background and Aims: Hepatitis C virus (HCV) infection is a leading cause of morbidity and mortality among HIV-positive men who have sex with men (MSM). Since 2000, there have been increasing reports of an epidemic of sexually transmitted HCV among HIV-positive MSM. Published incidence rates range from 0.5–4 infections per 100 person-years (py) among HIV-positive MSM, but work has indicated that since 2008 the incidence has stabilized or may now be falling. Previous studies in Amsterdam and London have also reported high HCV reinfection rates following treatment or spontaneous clearance (9–12/100py), however it is currently unknown whether HCV reinfection incidence has also fallen over this period.
Methods: We retrospectively identified all patients with a positive HIV and HCV antibody result from 2002–2012 at Chelsea and Westminster Hospital in London, UK. Primary HCV infections that were either successfully treated or spontaneously cleared were included in the analysis. HCV PCR results were followed through time calculating reinfection incidence for the time periods 2002–2008 and 2008–2014 using survival-time analyses.
Results: 191 HIV/HCV coinfected MSM were included in the analysis with a total of 589 years of follow-up. In total, 52 reinfections occurred between 2002–2014. From 2002–2008, 22 reinfections occurred at a rate of 13.2/100py (95% CI 8.7–20.1) compared to the period 2008–2014 where 30 reinfections occurred at a rate of 5.3/100py (95% CI 3.7–7.6) (Figure 1).
Conclusions: Among HIV/HCV coinfected MSM in London, there has been a statistically significant fall in HCV reinfection incidence over the past decade. This may be due to increased awareness of HCV transmission among patients and a concomitant reduction in risk behaviour. Reductions may also be due to increased uptake of screening and early treatment in this population preventing onward transmission. Future work should include analysis of reinfection over time in other cohorts, modelling of the epidemic to elucidate effective interventions and an analysis of risk-behaviour changes over time.

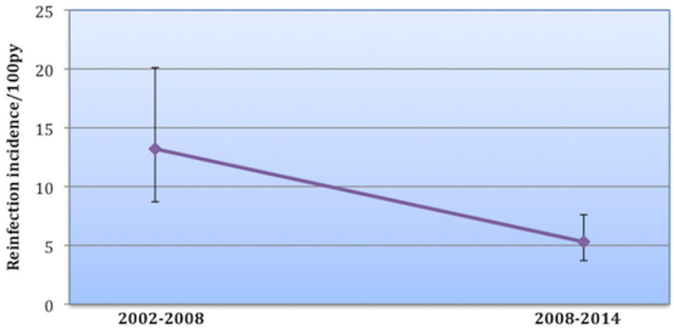

Figure 1.

P0724

DIRECT MEDICAL COSTS ASSOCIATED WITH THE EXTRAHEPATIC MANIFESTATIONS OF HEPATITIS C INFECTION IN THE UNITED STATES

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Background and Aims: Hepatitis C virus (HCV) infection is a systemic disease which is associated with both hepatic and extrahepatic manifestations. Although disease burden associated with the hepatic manifestation of HCV is well documented, the economic impact of the extrahepatic manifestation (EHM) of HCV [mixed cryoglobulinemia vasculitis (MCV), porphyria cutanea tarda (PCT), lichen planus (LP), type 2 diabetes (DM), depression (DD), chronic renal disease (CRD), and end-stage renal disease (ESRD)] remains unknown. The aim is to estimate the direct medical costs associated with the EHM of HCV in the United States.
Methods: We estimated the annual direct medical cost associated with EHM of HCV. The prevalence of each EHM in HCV was determined from an exhaustive review of recent literature (2005–2014). For the manifestations with definitive casual association, the actual HCV rates were used while for conditions without definitive causal association with HCV, the general population rates were subtracted. Outpatient resource utilization for HCV patients with different EHM were obtained from literature and the expert opinion. Inpatient utilization and per-patient-per-year (PPPY) inpatient and outpatient costs were estimated from Medicare billing data (adjusted to the 2014 U.S. dollars). Prescription drug costs were taken from literature or from the Red Book Drug Reference.
Results: In 2014, 2.68 million Americans were infected with HCV. The prevalence rates for the extrahepatic conditions in HCV-infected patients were as follows: MCV 7.5%, PCT 3.0%, LP 4.3%, CRD 4.8% (general population 1.3%), ESRD 0.4% (general population 0.3%), DM 14.8% (general population 9.3%), and depression 23.9% (general population 14.4%). The total PPPY costs associated with inpatient, outpatient and drugs ranged between $127 for LP to $29,000 for ESRD. To estimate the total annual economic burden for each extrahepatic manifestation, the respective prevalence rates were multiplied by PPPY costs. Thus, the total annual direct medical cost associated with the care of patients with extrahepatic manifestation of HCV in the U.S. was estimated to be $1,447 million per year. The sensitivity analyses (±25% to prevalence rates or ranges taken from the literature) suggested a range of $533 million to $2,618 million in total cost associated with extrahepatic manifestation of HCV.
Conclusions: Extrahepatic manifestations of HCV are quite costly and substantially add to the overall economic burden of HCV infection.

P0725

LIPIDOMICS ANALYSIS OF FASTING SERUM IDENTIFIES NOVEL LIPID BIOMARKERS SPECIFIC FOR HCV GENOTYPE 3 AND GENOTYPE 1 CHRONIC HEPATITIS C VIRUS INFECTION

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Background and Aims: HCV genotype 3 (G3) is now considered the most difficult to treat genotype in the era of new oral direct acting antiviral (DAAs) drugs, particularly in those with advanced liver disease. HCV-G3 is also associated with more rapid progression to cirrhosis. Evidence suggests that HCV-G3 has different effects on lipid metabolism compared to HCV-G1, associated with hepatic steatosis, low LDL cholesterol and distinct interactions in the formation of lipoviral particles. A lipidomic analysis was performed to identify lipid species differentially regulated in HCV-G3 compared to HCV-G1, to gain further insight into genotype-specific metabolic pathways.
Methods: Lipidomic analysis was performed on fasting sera [N = 112 (73 G1, 39 G3); 25% cirrhosis] using a UPLC system coupled to a QToF mass spectrometer. Profiles were acquired in positive and negative ion modes. System suitability and stability was confirmed by injection of a quality control (QC) sample at regular intervals throughout the analytical run, prepared by combining equal aliquots of all the study samples. Data was pre-processed (alignment, noise filtering, normalisation) using XCMS and in-house developed scripts, and subjected to multivariate statistical analysis using Simca-P. Principal component analysis (PCA) and orthogonal projection on latent structures-discriminant analysis (OPLS-DA) were performed on all data after pareto scaling.
Results: Spectra were explored by PCA for initial visualisation to detect inherent trends and outliers. Samples clustered based on the differences between the HCV-G1 and HCV-G3. Pairwise analysis using OPLS-DA allowed establishing the lipids with the strongest contribution to the genotype separation in positive ion mode. Preliminary assignment based on mass, fragmentation pattern and retention time of lipid species upregulated in HCV-G3 included *Cholesteryl linoleate* [M+NH4] 666.621 m/z @ 15.47 min. Other lipid species were specifically increased in HCV genotype 1 [M+H] 784.588 *m/z* @ 6.29 min *PC (36:30)*. In negative ion mode, additional novel lipid species were found to be differentially unregulated in HCV-G1. The novel lipid species are currently being identified and evaluated in an independent validation cohort.
Conclusions: UPLC MS lipidomics methods identify novel lipid species differentially regulated in HCV-G3 compared to HCV-G1. Understanding of the lipidome in HCV-G3 may help identify factors associated with DAA relapse.

P0726

DISCREPANCIES OF DATA ON THE EXISTENCE OF COMPLEX NATIONAL APPROACH TO HEPATITIS C TREATMENT DERIVED FROM GOVERNMENTAL, PROFESSIONAL/CIVIL SOCIETY AND PATIENT INSTITUTIONS IN 33 EUROPEAN COUNTRIES

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Background and Aims: In the era of safe and highly effective hepatitis C virus (HCV) infection treatment the management of HCV infected patients including national strategies and HCV treatment guidelines should become a healthcare priority in all European countries. The aim of the study was to search for potential discrepancies between governmental, civil society/professional and patient institution replies regarding the existence of complex national approach to HCV treatment.
Methods: For individual European countries governmental data on existence of HCV national strategies, clinical treatment guidelines and availability of publicly founded HCV treatment were extracted from the 2012 WHO Global Policy Report. They were compared to data given by highly motivated civil society and professionals (22 non-governmental, 7 public health and 4 university institutions) derived from the 2013 survey of 33 European countries (33 ECS). Data on governmental funding of hepatitis strategy and adherence to the European Association for the Study of the Liver (EASL) clinical treatment guidelines were derived from the 2012 Euro Hepatitis

Index Report performed by the European Liver Patient Association (ELPA) and compared to data of the two previous sources.
Results: Comparing WHO Report and 33 ECS, replies on existence of national strategy on viral hepatitis were almost identical, except for 1 country where the government reported on having national strategy which was in discordance to the civil society report, and for 2 countries with the government reports on no strategy whereas the professionals confirmed its existence. WHO Report on existence of national HCV treatment guidelines showed some discrepancies to 33 ECS in which in the opposite to governmental institutions the civil societies of 5 countries denied and of 3 countries confirmed their existence. 6 out of 8 countries that by ELPA reported high extent of adherence to EASL clinical guidelines, reported by 33 ECS to have national HCV treatment guidelines. According to WHO HCV treatment was publicly funded in all countries but 1 whereas patients institutions in only 11 countries reported access to publicly founded treatment as completely satisfactory.
Conclusions: Discrepancies in replies between governmental institutions and highly motivated civil society/professional and patient institutions, found in the study, may help creating future management of HCV infection in European countries.

P0727
CLINICAL UTILITY OF THE ARTUS HCV QS-RGQ ASSAY FOR HCV RNA QUANTIFICATION

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Background and Aims: Sensitive detection and accurate quantification of HCV RNA is essential for monitoring and determination of response to antiviral therapy. In this study we evaluated the clinical performance of the recently developed artus HCV QS-RGQ assay (artus HCV; Qiagen, Hilden, Germany).
Methods: A set of clinical samples representing HCV genotypes 1–4 (n = 108) was tested by artus HCV in comparison to COBAS AmpliPrep/COBAS TaqMan (CAP/CTM; Roche, Pleasanton, CA, USA) and Abbott RealTime HCV (ART; Abbott, Des Plaines, IL, USA). In addition, baseline and on-treatment (week 4, 8, 12) samples (n = 88) from patients with HCV genotype 1 infection treated with telaprevir-based triple therapy were also tested by all three assays.
Results: Overall, artus HCV showed good agreement with CAP/CTM and ART across all genotypes tested (mean difference 0.47 and 0.56 log10 IU/ml HCV RNA). The mean difference between artus and CAP/CTM in samples harbouring genotypes 1, 2, 3 and 4 was 0.37, 0.50, 0.40, and 0.60 log10 IU/ml HCV RNA, respectively. The mean difference between artus and ART for genotypes 1, 2, 3 and 4 was 0.50, 0.45, 0.58, and 0.67 log10 IU/ml HCV RNA, respectively. Analyses of on-treatment samples showed that overall concordance between artus HCV and CAP/CTM to classify samples as HCV RNA undetectable or below 25 IU/ml vs. detectable was 99%. Only one week 4 sample was HCV RNA positive by CAP/CTM but negative by artus HCV. Concordance between artus HCV and ART was 95% and only 3 samples collected at week 4 were positive by ART and negative according to artus HCV.
Conclusions: Artus HCV showed good agreement with CAP/CTM and ART for the quantification of HCV-RNA across genotypes 1–4. Overall, HCV RNA levels were approx. 0.5 log10 IU/ml higher according to artus HCV when compared to CAP/CTM and ART. Concordance of on-treatment samples obtained at major decision time-points (week 4, 12) was high between artus HCV and both comparator assays.

P0728
INVESTIGATION OF GENETIC RECOMBINATION OF HEPATITIS C VIRUS IN PATIENTS INFECTED WITH GENOTYPES 1, 2 AND 4

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Background and Aims: Genetic recombination is important for the evolution of RNA viruses. Different from other viruses for hepatitis C virus (HCV), only very few natural inter- and intragenotypic recombinant strains were reported. Recently, a putative recombination point was found within the NS2/NS3 junction and the identified inter-genotypic recombinants identified at a frequency of 0.8–1.4% in U.S. patients consisted of genotype (GT) 2 in the 5′ and GT1 in the 3′ part which led to determination of GT2 by standard Lipa genotyping assay and exhibited a reduced response to sofosbuvir + ribavirin treatment. Frequencies of recombinant HCV strains in European HCV genotype 1 and 2 isolates as well as HCV genotype 4 isolates from Egypt with evolution to a large number of subtypes is unknown.
Methods: Serum of patients chronically infected with HCV genotype GT1 from a German multicenter study (n = 162) and of consecutive patients infected with HCV genotype 2 (n = 58) and 4 (n = 121) who presented at University Hospitals in Frankfurt, Homburg and Cairo were investigated. To differentiate geno- and subtype recombinants according to the previously described NS2-NS3 breaking point, we amplified part of the core and NS5A or NS5B genes in GT1/4- and GT2-infected individuals with genotype-specific primers. Obtained PCR products were population-based sequenced and geno- and subtypes were determined using BLAST (HCV sequence database).
Results: Using sequence-based analyses, intragenotypic recombinants were investigated in patients with GT1 and 4 infection. In GT1, 47% (n = 76) of patients were infected with subtype 1a and 53% (n = 86) with 1b and no discordant subtypes or genotypes were observed between core and NS5A genes. In GT4-infected individuals, subtype 4a was most prevalent (87%, n = 106), followed by 4n (9%, n = 11) and the minor subtypes 4l and 4o (each 2%, n = 2) and all sub and genotypes were consistent between core and NS5A. Patients with GT2 were infected with subtype 2b (48%), 2a (26%), 2c (24%) and 2i (2%) and geno- as well as subtypes were in accordance between core and NS5B genes.
Conclusions: No inter- or intragenotypic HCV recombinants were detected in a cohort of >200 patients with chronic HCV genotype 1 or 2 infection in Europe which makes wrong selection of DAA-based antiviral therapy unlikely. Despite the large number of existing genotype 4 subtypes also no recombinants have been detected in genotype 4 infected patients by means of population based sequencing in the present study.

P0729
RENAL DISEASE PREVALENCE AND ASSOCIATION WITH HEPATIC FIBROSIS MEASURED BY FIB4 SCORE AMONG US PATIENTS WITH CHRONIC HEPATITIS C VIRUS (HCV) INFECTION IN THE CHRONIC HEPATITIS COHORT STUDY (CHECS)

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Background and Aims: Chronic HCV infection has been associated with several types of renal disease as both a causative and a

complicating factor; patients with severe renal manifestations of chronic HCV are at high priority for HCV treatment. We sought to describe the extent of renal disease and its relationship with other comorbidities and hepatic fibrosis among patients participating in the Chronic Hepatitis Cohort Study (CHeCS), a population-based prospective, observational cohort study at four large US health systems.

Methods: We observed HCV-monoinfected patients with renal and hepatic laboratory data, beginning from patient's HCV diagnosis in the health system through the end of 2012, with censoring at one year before any of the following events: death, loss to follow-up, sustained viral response to HCV therapy, or liver transplant. Hypertension (HTN), diabetes mellitus (DM), and chronic renal disease were defined by the presence of two ICD9 codes for the conditions at least 30 days apart. Generalized estimating equations were used to measure the correlation of impaired renal function (defined as estimated glomerular filtration rate [eGFR] <60) with level of hepatic fibrosis (assessed by FIB4 score) estimated from laboratory testing that occurred on the same day.

Results: Among 8,179 persons with mean follow-up of 7.5 years, the diagnosis of vasculitis, nephrotic syndrome and cryoglobulinemia was low: 0.3%, 0.6% and 0.8%, respectively. About one-half (53%) of patients were diagnosed with either DM or HTN; these patients were more likely to have the following renal disease conditions compared to those without HTN or DM ($p < 0.001$ for all, chi-square test): chronic kidney disease (16% vs 1%), eGFR <60 (19% vs 6%), and dialysis (4% vs 1%). Among patients with DM or HTN, the percent with eGFR <60 did not differ by FIB4 score categories. However, among patients without DM or HTN, the percent having eGFR <60 increased from 4% in those with FIB4 <1.6 to 10% in those with FIB4 ≥2.5 (Table). In a multivariate analysis, FIB4 ≥2.5 was associated with the risk of having eGFR <60 (adjusted OR = 1.5; 95% CI, 1.2, 1.8), after adjusting for age and other factors.

Table: eGFR levels by FIB4 score categories, CHeCS patients (N = 8,179)

eGFR levels	FIB4 score, n (%)		
	<1.6	1.6–2.5	≥2.5
Persons with HTN or DM			
≥90	589 (40)	418 (43)	863 (40)
60–89	602 (41)	378 (39)	865 (41)
45–59	90 (6)	86 (9)	201 (9)
30–44	57 (4)	36 (4)	119 (6)
<30	137 (9)	62 (6)	86 (4)
Persons without HTN or DM			
≥90	1075 (54)	350 (46)	667 (49)
60–89	842 (42)	359 (47)	569 (41)
45–59	56 (3)	38 (5)	101 (7)
30–44	12 (1)	7 (1)	22 (2)
<30	12 (1)	6 (1)	14 (1)

Conclusions: The prevalence of renal conditions associated with HCV infection such as cryoglobulinemia was low. In those without HTN or DM, advanced liver fibrosis measured by FIB4 score was significantly associated with high prevalence of impaired renal function (decreasing eGFR).

P0730

PREVALENCE AND CHARACTERISTICS OF HEPATITIS C GENOTYPES IN THE CHRONIC HEPATITIS COHORT STUDY (CHECS)

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Background and Aims: We sought to determine the prevalence of hepatitis C (HCV) genotypes (GT) /subtypes (ST), including demographics, disease characteristics, and death rates in the Chronic Hepatitis Cohort Study (CHeCS), an ongoing, observational cohort study which draws patients from four large and diverse US health systems.

Methods: Demographics, HCV GT/ST, viral load, aminotransferases, platelet count, FIB4, HBV/HIV coinfection, history of antiviral therapy, attainment of sustained viral response (SVR), development of hepatic decompensation and liver transplant were collected at last follow-up (date of last encounter or last antiviral therapy). We studied the prevalence of HCV GT/ST and compared patient and disease characteristics among non-deceased patients using the chi-square test. GTs 4–6 were grouped together due to small numbers. Death rates per 1000 person-years (p-y) were calculated and compared by Poisson regression.

	Genotype (%)					
	1 n = 1695	2 n = 801	3 n = 647	4 n = 102	5 n = 1	6 n = 19
Subtype						
a	56.7	6.1	94.9	10.8	0.0	47.4
b	30.9	62.3	0.5	0.5	0.0	0.0
Other/mixed	1.4	8.5	0.3	37.3	0	15.8
Not reported	10.9	23.1	4.3	52.0	100.0	36.8
				4–6 Combined		
Age						
≤40 years	11.5	10.0	19.6	21.3		
40 ≤ 50 years	16.3	21.7	26.1	21.3		
50 < 60 years	48.2	45.2	43.9	38.5		
≥ 60 years	24.0	23.1	10.4	18.9		
Male gender	60.3	54.6	60.6	45.9		
Race						
Asian	3.0	4.0	3.9	21.3		
Black	29.6	8.4	2.9	7.4		
White	60.7	79.5	85.0	59.8		
Other	3.4	3.2	3.2	1.6		
Unknown	3.3	4.9	4.9	9.8		
FIB4						
≤1.21	27.4	35.3	33.7	37.7		
1.21 ≤ 5.88	57.4	55.1	50.2	41.8		
>5.88	12.8	7.2	13.8	16.4		
Missing	2.4	2.4	2.3	4.1		
ALT						
<LLN and/or Normal	59.1	69.8	57.3	59.8		
ULN ≤ 2×ULN	26.9	19.5	21.8	27.0		
>2×ULN	13.4	10.5	20.2	12.3		
Unknown	0.6	0.2	0.6	0.8		
Decompensated	12.2	5.2	12.8	9.8		
Viral load						
Indeterminate	6.7	4.2	3.3	4.8		
Undetectable	18.1	35.7	38.5	32.3		
Detectable, ≤500,000	24.5	23.0	27.3	41.9		
Detectable, >500,000	50.7	37.1	30.9	21.0		
Liver transplant	5.1	2.2	7.9	4.1		
HBV co-infected	0.6	0.9	1.4	0.8		
HIV co-infected	3.2	2.0	1.7	4.1		
Treatment experienced	43.3	50.3	53.0	51.6		
SVR attained	22.5	53.1	46.6	36.5		

Results: 11,111 patients were included, of whom 9106 (82%) were alive; median follow-up was 6.7 years. Genotype data were available for 6265 (69%) patients. The table shows GT/ST prevalence and characteristics among non-deceased patients. The chi-square test showed significant differences among the GTs in all demographic and disease variables ($p < 0.001$) except HBV/HIV coinfection ($p = 0.10$ and 0.06). In general, GT2 patients have lower ALT, FIB-4, and rates of liver transplant and decompensation than GTs 1 and 3. Viral loads are higher in GT 1 than in GTs 2, 3 and 4–6. Fewer GT1 patients received antiviral therapy than others. SVR responses were 2-fold or higher in patients with GT 2 or 3

compared to those with GT 1. Blacks were dominantly GT 1 (30%), compared to other GTs (3%>8%). Death rates were 31.6, 25.7, 28.8 and 18.7 per 1000 p-y for GTs 1, 2, 3 and 4–6 respectively (p = 0.0214).

Conclusions: Our current real-world US data (up to January 1, 2013) showed 75% patients were infected with G1, among those 57% and 31% were subtype 1a and 1b respectively. Significant differences were observed among GT1, 2 and 3 in patients' race, fibrosis biomarkers, treatment allocation and response to treatment. Patients with GT2 and 3 had lower HCV RNA levels, higher likelihood of receiving therapy and higher SVR compared to GT1.

P0731

LEDIPASVIR (LDV)/SOFOSBUVIR (SOF) TREATMENT OF HEPATITIS C VIRUS (HCV) IS ASSOCIATED WITH REDUCTION OF SERUM APOLIPOPROTEIN LEVELS

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Background and Aims: The interaction of lipoproteins with HCV has been of pathogenic and therapeutic interest. It is postulated that HCV circulates in the blood, bound to lipoproteins such as very-low-density lipoprotein, which potentially plays a role in its ability to escape immune clearance. HCV also potentially uses lipoproteins receptors to gain access into hepatocytes. Our aim is to evaluate changes in the apolipoprotein profile of patients with chronic hepatitis C (CH-C) during and after successful cure with LDV/SOF±ribavirin (RBV).

Methods: CH-C genotype 1 patients who had achieved SVR-12 after treatment with 12 weeks of LDV/SOF±RBV were selected from the ION-1 clinical trial. Frozen serum samples from baseline, end of treatment and 4 week follow-up were used to assay apolipoproteins (Apo A1, Apo AII, Apo B, Apo CII, Apo CIII, Apo E) using the Multiplex platform. Changes in serum apolipoprotein level (mcg/mL) from baseline levels were assessed at the end of treatment and after 4 weeks of follow-up.

Results: 100 CH-C patients were included (mean age 53 years, 57% male, 17% cirrhosis, 73% genotype 1a). At the end of treatment, a significant reduction in Apo AII levels (−14.97±63.44 mcg/mL, p = 0.0067) and Apo E levels (−4.38±12.19 mcg/mL, p < 0.001) was noted. These declines in Apo AII (−16.59±66.15 mcg/mL, p = 0.0075) and Apo E (−2.66±12.64 mcg/mL, p = 0.01) persisted after 4 weeks of follow-up. On the other hand, Apo CII level increased from baseline to end of treatment (2.74±11.76 mcg/mL, p = 0.03) and persisted after 4 weeks of follow-up (4.46±12.81 mcg/mL, p = 0.0005). However, this increase in Apo CII during treatment was observed in patients receiving RBV-free SOF/LDV only (5.52±11.92 mcg/mL, p = 0.0007), while no similar increase was noted in SOF/LDV+RBV (p = 0.64). In the multivariate analysis, treatment with LDV/SOF+RBV was independently associated with lower levels of all lipoproteins at the end of treatment. In particular, the reduction in Apo E associated with RBV-containing treatment (compared to RBV-free SOF/LDV) was −5.31 mcg/mL (p < 0.05).

Conclusions: Treatment with LDV/SOF±RBV is associated with a persistent reduction in the apolipoprotein AII and E, as well as with an increase in Apo CII after achieving cure. Adding RBV is independently associated with reduction in Apo E, and essentially eliminates the increase in Apo CII. These data suggest that LDV/SOF±RBV may overcome this mechanism of escape for HCV.

P0732

IMPACT OF OLD SCHISTOSOMIASIS INFECTION ON THE USE OF FIBROSCAN FOR STAGING OF FIBROSIS IN CHRONIC HCV PATIENTS

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Background and Aims: *Background:* Diagnosis of the stage of liver fibrosis is essential for deciding an antiviral therapy. Multiple non invasive methods for assessment of liver fibrosis as fibroscan have been studied. HCV-Schistosomiasis coinfection is more common among Egyptian patients.

Aim: Assess the impact of Schistosomiasis on the use of fibroscan for staging of fibrosis in chronic HCV patients.

Methods: This study was conducted on 326 chronic HCV patients. Routine laboratory workup was done and serum Antischistosomal antibody using ELISA technique was performed. Reference needle liver biopsy was histopathologically assessed based on Metavir scoring for staging of fibrosis (F0–F4) and Liver stiffness measurement by FibroScan® was done for all the patients.

Results: Antischistosomal antibody was positive in 59.3% of the studied population. The disagreement between the results of liver biopsy and fibroscan was more obvious in those with positive schistosomal serology with p value 0.08 (approaching significant). Although Periportal tract thickening was found in 24% of the studied population, it showed no impact on the agreement between liver biopsy results and fibroscan (p value 0.22). Multivariate logistic regression analysis showed that high BMI (≥30) and positive anti schistosomal antibody were the most important factors associated with disagreement between the results of fibroscan and liver biopsy especially in F3 group with p values (0.023 and 0.029 respectively)

Conclusions: Performance of fibroscan for staging of fibrosis may be impaired by the presence of schistosomiasis coinfection.

P0733

DIABETES MELLITUS INCREASES THE RISK OF LIVER CIRRHOSIS AND HCC IN SAUDI HCV PATIENTS

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Background and Aims: *Background:* Several epidemiological studies have suggested an association between type II DM and HCV infection. Some other studies have shown a lower viral response to treatment in diabetic patients and higher incidence of HCC. Few reports from Saudi Arabia demonstrated a higher prevalence of DM in HCV infected patients but the association with liver cirrhosis, response to treatment and development of HCC is not known.

Aims: To assess the impact of the prevalence of Diabetes mellitus in HCV infected patients on the risk of development of liver cirrhosis and hepatocellular carcinoma.

Methods: Retrospective chart review of patients diagnosed with HCV infection between 2002 and 2012. patients were identified from an institutional database for hepatology patients. Demographic, clinical, histological, biochemical and serological data were collected. Variables were analyzed descriptively and inferentially using SPSS.

Results: Retrospectively we evaluated 392 patients infected with HCV. The mean age of this cohort was 51.6±13.6 years and 51% were males. Genotype was available for 50% of the patients. Genotype 4 was the commonest (56%) and genotype 1 was found in 32.3% while the rest (11.7%) had genotype 2, 3, 6 or mixed genotype. The

prevalence of diabetes was 47.8%. There was no difference in BMI between diabetic and non diabetic patients (p 0.139). Histology data were available for 232 patients (59%). At presentation, 31.3% of the patients had early (stage 0–2) fibrosis and 27.2% had advanced (stage 3–4) fibrosis. Diabetic patients were more likely to have advanced fibrosis (53.3% vs 42.3%) but difference was not statistically significant, p 0.096. Cirrhosis occurred more frequently in diabetic patients (OR 1.98, p 0.001). Similarly, HCC was more prevalent in HCV infected patients with diabetes (OR 1.89, p 0.056) and the mortality rate was higher (p 0.033). Treated patients with diabetes had less SVR (OR 0.63) however the difference was statistically insignificant (p 0.106).
Conclusions: Diabetes mellitus is highly prevalent in HCV infected patients. This was independent of obesity in this population. HCV infected patients with diabetes tend to have less response to antiviral therapy, higher incidence of cirrhosis and HCC and higher mortality rate compared to those without DM.

P0734
HEALTHCARE UTILISATION FOLLOWING TREATMENT OF HIV/HCV PATIENTS WITH MILD LIVER DISEASE

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Background and Aims: New directly acting antiviral (DAA) therapy for hepatitis C (HCV) offers the potential for high cure rates in many patient groups previously considered difficult-to-treat, including those HIV/HCV co-infected. However, the high price of these medications is likely to limit access to treatment in all healthcare settings, at least in the short term. Early treatment priority is likely to be given to those with advanced disease but there are potential benefits in treating those with mild disease including the reduction of transmission and reduction HCV-related healthcare utilisation. Benefits in healthcare utilisation are likely to differ between mono- and HIV co-infection as HIV patients remain within healthcare services. We hypothesised that successful HCV treatment within a HIV/HCV co-infected population with mild liver disease would lead to a reduction in the HCV-related use and costs of healthcare services in the 5 years following treatment completion.
Methods: In a retrospective analysis, HIV/HCV co-infected patients that completed a course of therapy between January 2004 and March 2014 at St. Mary's Hospital were evaluated, excluding those with a fibroscan ≥9.6 kPa and/or a biopsy with ISHAK score ≥2/6 in the 2 years prior to treatment. Detailed analysis of healthcare utilisation up to 5 years following treatment for each patient was conducted using electronic records and subsequent costs were calculated.
Results: 63 patients with mild disease completed a course of HCV therapy between January 2004 and March 2014, of which 48/63 (76%) attained SVR12. Both SVR and non-SVR groups were predominantly white males with a mean age of 46 and 41, respectively. Genotype 1 was the most prevalent, present in 33/48 (69%) SVR and 11/15 (73%) non-SVR patients. Individuals that failed treatment were more likely to spend at least one night in hospital in the 5 years following therapy with a RR of 4.00 (p = 0.0213, 95% CI, 1.23–13.02). Overall, patients achieving SVR12 incurred lower health utilisation costs (£3,712.20 per patient) compared to (£9,299.70 per patient) non-SVR patients.
Conclusions: Healthcare utilisation rates and costs in the immediate 5 years following treatment were significantly higher in co-infected patients with mild disease that failed to achieve SVR12 compared to those who successfully cleared the virus. This data suggests additional value to achieving cure beyond the prevention of complications of disease.

P0735
ASSESSMENT OF BASELINE VIRAL LOAD CUT-OFF FOR SHORTENED LEDIPASVIR/SOFOSBUVIR THERAPY BY WIDELY USED HCV RNA ASSAYS

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Background and Aims: The recent approval of ledipasvir/sofosbuvir combination therapy for the treatment of HCV genotype 1 infection includes the possibility to shorten treatment duration from 12 to 8 weeks in non-cirrhotic treatment-naïve patients who have a baseline viral load (VL) <6 million IU/ml (i.e. 6.8 $\log_{10}$ IU/ml). In this multicentre study we assessed the reliability of this new cut-off with three commercially available HCV RNA assays.
Methods: Baseline samples from patients enrolled in the m11-206 phase 2 study (n = 73) as well as patients referred to our outpatient clinics (n = 148) were tested for HCV RNA with the Roche HighPureSystem Cobas TaqMan (HPS/CTM) or Cobas Ampliprep/Cobas TaqMan (CAP/CTM) and Abbott RealTime HCV (ART) assays. All patients were non-cirrhotic and naïve to antiviral therapy, thus in principle eligible for an 8-week regimen. In a subset of patients (n = 48 with CAP/CTM; n = 12 with ART) with baseline VL >1 million (6.0 log) IU/ml, samples from 1–2 additional screening time-points were available for repeated HCV RNA testing.
Results: Of the m11-206 study baseline samples tested, 44/73 (60%) and 7/73 (10%) had >6 million (6.8 log) IU/ml according to HPS/CTM and ART, respectively. Among patients referred to our clinics, 20/148 (14%) had >6 million IU/ml by CAP/CTM. Of the 75/148 outpatient samples tested with both CAP/CTM and ART, 6 (8%) and 2 (3%) had >6 million IU/ml. In patients with repeated pre-baseline measurements clinical decisions could be impacted in 29% of cases for CAP/CTM due to variability in the measured HCV RNA level (above and below 6 million IU/mL). Eight patients could have shortened and 5 extended treatment depending on the time point considered for treatment decision. In contrast, only one patient with a baseline VL <6 million IU/ml according to ART would have received extended therapy based on a VL >6 million IU/ml at a preceding visit.
Conclusions: A baseline viral load below 6 million (6.8 log) IU/ml was present in 59% of patients in the ION-3 registration trial and 40% in the present study based on HPS/CTM. However, measurements with CAP/CTM and ART, two assays widely used in the real-world setting, revealed that 86% and 90% of patients tested were, in principle, eligible to receive an 8-week treatment regimen. A substantial proportion of patients had fluctuating VL levels below and above 6 million IU/ml at different screening time points according to CAP/CTM that could potentially impact treatment decisions.

P0736
IMPACT OF PHYSICAL ACTIVITY ON OXIDATIVE STRESS IN PATIENTS WITH HEPATITIS C

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Background and Aims: The beneficial effects of increased physical activity (PA) on non-alcoholic fatty liver disease (NAFLD) in obese subjects have been reported recently. The goal of this study was to evaluate the effect on oxidative stress of moderate PA in patients with hepatitis C.

Methods: Patients with Hepatitis C (n = 13) were exposed to moderate PA for 12 weeks. An individualized program of aerobic exercise lasting 16 weeks in 3 sessions of 60 minutes/week was designed. Different clinical parameters were recorded during that period, including samples from whole blood. Serum and plasma sample were obtained and PBMC cells were isolated for further RNA extraction and gene expression analysis. Catalase activity was monitored at t = 0 (T0; baseline), t = 6 weeks (T6) and t = 12 (T12) weeks using the Catalase Assay kit (Cayman Chemicals, Michigan USA). PBMC were isolated for t = 0 and t = 6 weeks. Superoxide dismutase 1 (SOD1), SOD2 and Nitric oxide synthase 2 (NOS2) gene expressions were analyzed. Statistical analysis was performed using SPSS v22.
Results: PBMCs are considered a good model to reflect important metabolic changes in the liver. Gene expression was analyzed in t = 0 and t = 6 weeks samples from HCV patients. 30% of patients showed reduced expression of NOS2 and SOD2 after 6 weeks of PA (fold inhibition 31±4.3; $p < 0.05$). SREBP1c, which plays a pivotal role in lipid metabolism, was also found down-regulated in the T6 group compared to T0 (27±3.3 fold inhibition). Mean values of catalase activity was analyzed in T0, T6 and T12 groups. Difference in enzyme activity was not significant when T0 and T6 groups were compared (t test) (20.37±1.32 vs. 22.44±1.84; p = ns). However, significant differences were found between T0 and T12 groups (20.37±1.32 vs. 27.33±1.94; $p = 0.005$) and between T6 and T12 groups (22.44±1.84 vs. 27.33±1.94; $p = 0.003$).
Conclusions: A common pathogenic feature present in HCV infection is oxidative stress. We have demonstrated that physical exercise improves antioxidant state in patients with HCV through regulation of catalase activity and the expression of oxidative stress-related genes (SOD2 and NOS2). Hence, moderate PA is recommended for patients with HCV.

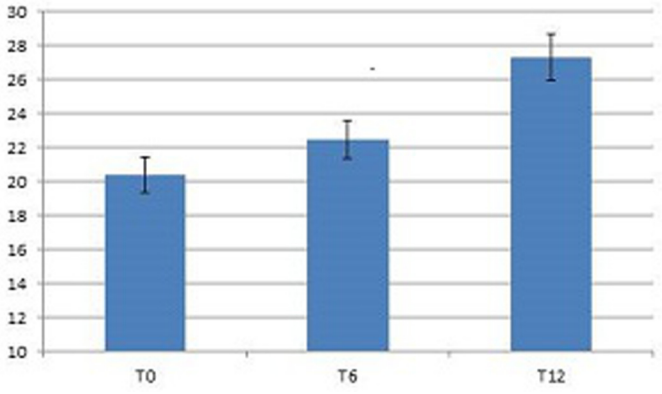

Figure 1. Catalase activity (in relative units) in HCV patients at baseline (T0), 6 weeks (T6) and 12 weeks (T12) of physical activity.

P0737
MELD SCORE KINETICS AND SURVIVAL IN HIV/HCV PATIENTS: THE ANRS EP 25 PRETHEVIC COHORT

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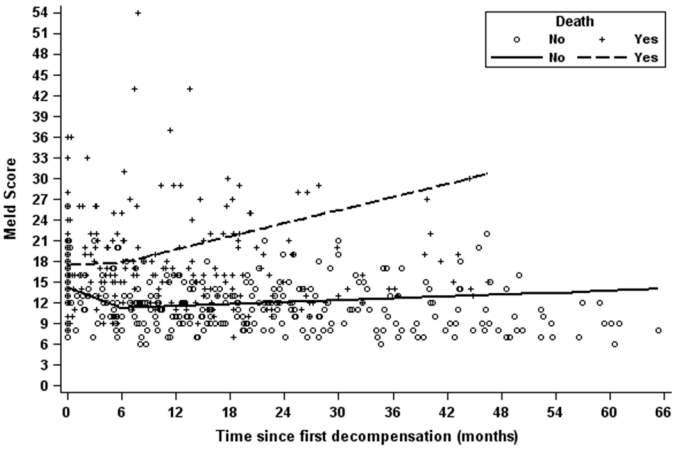
Background and Aims: HIV infection fastens liver disease progression in patients with hepatitis C virus infection. HIV/HCV coinfected patients have higher rates of hepatic decompensation and more severe liver events than HCV mono-infected patients. There are conflicting data concerning the predictive factors of mortality of patients with end stage liver disorder, particularly after the first episode of decompensation. It has been demonstrated that MELD score was an independent predictor of the pre-transplant mortality. The aim of this study was to assess prognostic factors of survival and describe longitudinal MELD dynamics in HIV/HCV co-infected patients who further died (D) or not (ND) after a first episode of liver decompensation (DC).
Methods: 67 HIV/HCV co-infected pts without hepatocellular carcinoma and with a first episode of DC within 1 year (yr) before enrollment were prospectively followed. Clinical and biological data were collected at 1st DC and then every 3 months. Survival rate and risk factors of death were studied by using Kaplan–Meier curves and Cox models. A two-slope mixed linear model with a node at 6 months (mths) was used to estimate change of MELD in D and ND from 1st DC.
Results: Patients were included in 32 centers in 2009–2013 (72% male, median age: 48 yrs [IQR 44–51], median follow-up: 23.4 mths [13.9–44.8]). The median number of recurrent episodes of DC was 2 [1–3] after 1st episode. Overall survival rate was 87%, 77% and 60% at 6, 12 and 24 mths after 1st DC, respectively. In multivariate analysis, MELD score at 1st DC was predictive of mortality, adjusted for age, sex, severity of 1st DC and time between HIV/HCV diagnosis and 1st DC: aRR was 1.32 [95% CI: [1.10–1.57], $p < 0.003$ for an increase of 3 units of MELD. The kinetics MELD scores (Figure) from 1st DC significantly differed between D and ND pts. In ND patients, mean MELD significantly decreased during the first 6 mths (−0.49/mth; $p = 0.02$), while it remained flat in D patients (+0.06/mth, $p = 0.75$). After 6 mths, mean MELD increased significantly by +0.32/mth ($p < 0.0001$) in D patients while it was only +0.05/mth ($p = 0.17$) in ND pts. Before and after 6 mths, the slopes significantly differed in D and ND subjects.
Conclusions: MELD is an effective tool to predict mortality in HIV/HCV co-infected patients with liver decompensation. An increase in MELD, 6 months after the 1st decompensation, should alert for a pre-transplant assessment. This is especially important in this poor prognosis population.



P0738
HEPATITIS C VIRUS INFECTION IN SUB-SAHARAN AFRICA: PREVALENCE META-ANALYSIS AND RISK FACTOR REVIEW

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Background and Aims: The Global Burden of hepatitis C working group estimates that the prevalence of Hepatitis C virus (HCV) infection is approximately 2.2% worldwide, with Sub-Saharan

Africa (SSA) region having among the highest prevalences. In Western countries the modes of transmissions are widely established. However, in developing countries, especially in SSA, the transmission pathways and risk factors are not well known.
The primary aim of the study was to conduct a meta-analysis on the HCV sero-prevalence in SSA, and examine the impact of region, cohort and year of study on the estimates. The second aim was to perform a literature review of risks factors for HCV infection in SSA.
Methods: Articles published from January of 2000 to December of 2013 on HCV prevalence and risks factors were assessed. Studies were excluded if they were missing information on prevalence, year of study, serologic assay used, or were not from sub-Saharan countries.
Study populations were grouped by cohorts based on different risk factors (i.e. general population, HIV positive, chronic illness, high risk, and blood donors).
Results: Data from 265 studies representing the SSA region were. A total of 146 articles meeting the inclusion criteria. Studies came from 26 countries across 4 sub-regions in SSA.
Overall, the prevalence estimate was 4.9% (95% CI: 1.9–12.0). The prevalence varied by region. There was a higher prevalence in studies from current years (4.4%), as opposed to earlier years (2.9%). Prevalence also varied by population cohort. Table 1 depicts the prevalence estimates by population cohort, region, and year of study. A paucity of data limited the possibility to conduct a meta-analysis on risk factors for HCV infection. However, risk factors consistently associated with HCV infection amongst others include: gender, traditional circumcision, ethnic group, history of past injections, HIV and HBV co-infection, education level, and occupation (farming).

Table: Prevalence of HCV infection in Sub-Saharan Region. Meta-analysis results [a]

Group	Prevalence, %	95% CI
Overall	4.9	0.2–12.0
Cohort		
General population	4.2	3.1–5.7
Blood donors	1.5	0.8–3.1
HIV positive	3.0	0.9–10.0
High risk population	11.8	1.7–79.0
Chronic illness	7.5	0.2–236.0
African Region		
Central	7.0	4.6–10.0
Eastern	3.0	1.5–5.7
Western	3.9	2.0–7.5
Southern	0.3	0.06–17.0
Year		
2000 to 2005	2.9	1.8–4.6
2006 to 2013	4.4	2.9–6.6

[a] Random effect model.

Conclusions: There is limited primary data on HCV prevalence. This meta-analysis, however, suggests that HCV prevalence has increased and is higher than previously reported estimates. There is even less data on HCV risk factors, limiting our understanding of present day transmission pathways. Such knowledge gaps limit the ability to create screening and treatment guidelines relevant to this region.

P0739
IMPACT OF SERUM APOLIPOPROTEIN PROFILES IN CHRONIC HCV INFECTION: DIFFERENCE BETWEEN HCV GENOTYPES 1B AND 2

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Background and Aims: The life cycle of hepatitis C virus (HCV) is tightly associated with host lipoprotein metabolic pathways. Many articles have demonstrated prominent lipid metabolism disturbances with HCV genotype 3a (G3a). Although HCV G3a infection is extremely rare in Japan, abnormalities of lipids/lipoproteins are recognized in Japanese HCV G1b/G2 patients. In order to clarify abnormality of lipoprotein in chronic HCV infection, we examined serum apolipoprotein profiles, because apolipoprotein is present on the outer surface of lipoprotein particles and plays a crucial role in lipoprotein metabolism.
Methods: Fasting serum apolipoprotein profiles of 358 subjects with active or cleared HCV infection were examined. Basic laboratory data including lipids were examined at the same time. Concentrations of apo A-I, A-II, B, C-II, C-III, and E were measured by immunoturbidimetric assay using a commercially available kit according to the manufacturer's instructions (Sekisui Medical Co., LTD, Tokyo, Japan). Subsequently, the association between chronic HCV infection and serum apolipoprotein levels was determined using multiple regression analysis.
To clarify the significance of the genotype (G1b or G2) of actively infected HCV patients on the serum level of apolipoproteins/lipids, similar multiple regression analysis using indicator variables was performed.
Results: Active HCV infection was associated with high serum levels of apo A-II ($p < 0.001$) and low serum levels of apo C-II ($p = 0.04$) and C-III ($p < 0.001$). In contrast, active HCV infection was not found to be a significant factor affecting any alteration of serum lipid levels. HCV infection with both G1b and G2 was associated with low serum levels of apo C-III ($p < 0.001$), whereas only HCV G1b infections caused high levels of apo A-II ($p < 0.001$) and low levels of apo B ($p = 0.047$) and C-II ($p = 0.04$). Additionally, IFNL3 rs8099917 non-major genotype (TG/GG) was associated with high levels of apoA-II and low levels of serum apo B in chronic HCV G1b infection.
Conclusions: Our results demonstrate that active HCV infection is distinctively associated with characteristic serum apolipoprotein profiles. The influence on apolipoprotein profiles varies with different HCV genotypes. Moreover, genotype of IFNL3 affected serum apolipoproteins in HCV G1b infection.

P0740
USEFULNESS OF INTERFERON LAMBDA4-, HLA CLASS II-POLYMORPHISM AND KILLER-IMMUNGLOBULIN-LIKE RECEPTOR-2DL3:C1/C1 TO PREDICT SPONTANEOUS CLEARANCE OF ACUTE HEPATITIS C VIRUS INFECTION

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Background and Aims: Spontaneous resolution of acute hepatitis C virus infection (AHC) largely depends on the patient's immunogenetic background. Besides IL-28B (rs12979860), Interferon-Λ4/rs469415590- and HLA-class-II/rs4273729 polymorphisms (PMs) as well as the detectability of killer-cell-immunoglobulin-like-receptor with its ligands (KIR)2DL3:HLA-C1/C1 might influence spontaneous clearance (SC) of AHC. We aimed to investigate if these genetic factors predict SC in patients with acute HCV-infection.
Patients and Methods: IL-28B/rs12979860-, IFNΛ4/rs469415590- and HLA-class-II/rs4273729 polymorphisms were determined in

163 patients [m/f: 97/66; age: 34.7±14.9 years (mean±SD); SC: N = 73]. Presence of KIR2DL3:C1/C1 was evaluated in 100 AHC-patients [m/f: 55/45; age: 35±15 years (mean±SD); SC: N = 61]. In addition 96 healthy individuals (healthy control [HCo]) were examined. IL28B-, interferon-Λ4- and HLA-class-II-PMs were analyzed by StepOnePlus-Real-Time-PCR. Presence of KIR2DL3:C1/C1 was determined by use of a commercial kit [HistoSpot, BAG, Lich, Germany].

Results: Since IFNΛ4-TT/TT and IL-28B C/C (rs12979860) genotypes were overlapping, both were equally predictive for spontaneous resolution of acute hepatitis C virus infection [IFNΛ4-TT/TT&IL28B-C/C: SC: N = 50/73 (68.5%) vs. non-SC: N = 45/90 (50%); P = 0.025]. Both SNP's were more frequent in patients with SC compared to the healthy controls [SC: 50/73 (68.5%) vs. HCo: 38/96 (39.6%); P < 0.001]. Neither the favorable HLA-II/rs4273729-genotype G/G [SC: N = 31/73 (42.5%) vs. non-SC: 29/90 (32.2%); P = 0.19] nor the detectability of KIR2DL3:HLA-C1/C1 [SC: N = 20/61 (32.8%) vs. non-SC: N = 10/39 (25.6%); P = 0.5] were found to be associated with SC.

Conclusions: Polymorphisms within IFNΛ4 or HLA-class-II as well as the detectability of KIR2DL3:C1/C1 could not improve predictability of SC in AHC-patients compared to the determination of IL28B/rs12979860 alone. Thus, their determination does not provide additional benefit to identify patients who will clear HCV spontaneously.

P0741

IMPACT OF PILL COUNT ON MEDICATION ADHERENCE DURING THE FIRST 12 WEEKS OF HIV ANTIVIRAL TREATMENT: IMPLICATIONS FOR HCV TREATMENT

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Background and Aims: The impact of pill count on adherence to the new all-oral antiviral hepatitis C (HCV) treatments has not been studied. In another therapeutic area where treatment consists of a combination of antiviral drugs – HIV – research has shown that pill count can negatively affect long-term adherence. However, unlike HCV where all-oral regimens generally involve only 12 weeks of treatment, ongoing treatment is required in HIV. To better understand the possible impact of pill count on adherence to a short-term HCV regimen, we examined the impact of pill count on adherence to the first 12 weeks of HIV treatment.

Methods: This retrospective study assessed real world adherence as proportion of days covered (PDC) over the first 12-weeks of treatment for HIV patients initiating first-line therapy. Three once-daily treatment regimens containing the same medications but in different formulations were examined:

- Cohort 1: Atripla (Efavirenz + Tenofovir + Emtricitabine); 1 pill per day,
- Cohort 2: Efavirenz + Truvada (Tenofovir + Emtricitabine); 2 pills per day,
- Cohort 3: Efavirenz + Tenofovir + Emtricitabine; 3 pills per day.

Analyses were conducted using MarketScan US commercial pharmacy claims and enrollment data (January 2003 through September 2013). Index date was first fill date for each drug combination. Inclusion criteria: continuous enrollment 6-month pre-index date and 3-month post-index date. Exclusion criteria: patients with a 90 day drug supply on initial fill, <18 years of age, and use of any HIV drug in the pre-index period or use of any other HIV drug besides cohort drugs in the post-index period. Generalized linear modeling was used to adjust adherence by age and gender. Statistical significance was defined as $p < 0.05$.

Results: Patients totaled 7411 for cohort 1, 562 for cohort 2, and 80 for cohort 3. Median age was 40, 41 and 40 years and percent male was 86%, 83% and 88% for cohorts 1, 2 and 3, respectively. The 12-week adjusted adherence rates were 83%, 81% and 81% in cohorts 1, 2, and 3, respectively. There was no statistically significant difference in adjusted adherence rates between any cohort. Male gender and older age were associated with better adherence.

Conclusions: These findings suggest that pill count does not appear to be a factor in antiviral medication adherence over the initial 12-weeks of treatment. As more all-oral HCV treatments become available, this research should be replicated in the HCV population.

P0742

HEPATITIS C TESTING IN PEOPLE ATTENDING AN INNER CITY DRUG MISUSE CENTRE – ARE SCREENING GUIDELINES ADHERED TO?

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Background and Aims: Worldwide 130–170 million people have chronic hepatitis C (HCV); annually 350,000 deaths occur secondary to HCV cirrhosis and hepatocellular carcinoma [1]. The advent of direct acting antivirals has revolutionised HCV treatment but identifying those infected remains a barrier to treatment and virus eradication. Guidance states that annual screening for HCV should be performed in people accessing drug services; however testing in this group remains haphazard. We aim to review HCV screening in a large inner city substance misuse centre to establish whether guidelines are adhered to.

Methods: We reviewed the electronic medical records of every service user (SU) still registered at the unit as of October 2014 to establish the timing and result of their last screening test. When screening had not been performed in the preceding twelve months, we looked for reasons in the records.

Results: 550 SU attended the unit; 455 males (M) (83%) [age range (AR) 21–64, median age (MA) 41], 95 females (F) (17%) (AR 23–61, MA 39). 520 (95%) SU had been screened at least once since 2006, 30 (5%) SU had never been screened. 335/520 (64%) SU (277M, 58F) were HCV antibody (Ab) negative, 133 (26%) SU (117M, 16F) were HCV Ab positive PCR positive, 51 (9.9%) SU (37M, 14F) were HCV Ab positive PCR negative and 1 (0.1%) SU was Ab positive PCR unknown. In the HCV Ab negative cases, 113/335 (34%) had undergone screening in the last 12 months; 107 (32%) were screened between months 13–24, 48 (14%) between 25–36 months and 67 (20%) had not been screened for more than 3 years. Review of the records revealed that in 140/222 (63%) cases no reason was documented for not having annual screening. In 81 (36%) cases screening was declined by the SU and in 12% the reason stated was 'SU considers themselves not at risk'. Failed venepuncture was documented in 1 case (0.4%).

In the HCV Ab positive patients, 21/52 (41%) had been tested within the last 12 months, 10 (19%) tested between months 13–24, 12 (23%) between months 25–36, 9 (17%) had not been tested for more than 3 years.

Conclusions: Despite clear guidelines on HCV testing, this study highlights that annual testing in SU is not performed. Re-education of staff, access to less invasive testing methods including dry blood spot and additional staff to support screening requirements may all help to adhere to guidelines

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P0743

INTERLEUKIN-6 IS ASSOCIATED WITH LIVER FIBROSIS IN HCV-INFECTED PATIENTS WITH ALCOHOL ABUSE

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Background and Aims: Both hepatitis C virus (HCV) and alcohol abuse cause chronic hepatic inflammation and alterations in serum inflammatory cytokines. The mechanisms by which alcohol worsens hepatitis C virus-related liver disease have not been fully clarified; however, impairment of host's immune response should be highlighted. In this context, the influence of cytokines on liver fibrosis needs to be well characterized. In this study, we investigated the association between interleukin (IL-6), IL-10, IL-17A and tumour necrosis factor (TNF-α) and liver fibrosis in HCV-infected patients with current or past alcohol abuse.

Methods: A total of 130 consecutive patients (73 females; mean age, 52.6±11.6 years) with chronic hepatitis C (CHC) were enrolled in the study. All patients completed several surveys including semi-structured interview, Mini-International Neuropsychiatry Interview (MINI-Plus 5.0) and CAGE screen (defined as two or more affirmative answers). The amount, frequency, and duration of use of alcohol were evaluated in CHC patients (alcohol abuse was defined as >20 g/day for females and >40 g/day for males). The diagnosis and staging of the underlying liver disease was based on standard clinical, biochemical, serological, radiological and histological parameters. The serum levels of IL-6, IL-10, IL-17A and TNF-α were determined by Cytometric Bead Array Assay. The protocol was approved by Ethical Board of UFMG. Data were analyzed with SPSS 17.0. Linear and logistic regression models were used to assess the association between cytokines and cirrhosis, adjusting for age, sex, body mass index and alcohol use.

Results: Baseline characteristics were: 102 (78.5%) with CHC and 28 (21.5%) with compensated cirrhosis; 43 (33.1%) with alcohol abuse. Higher levels of IL-6 was associated with alcohol abuse ($\beta = 0.43$; $t = 3.14$; $p = 0.003$) and hepatic necroinflammatory activity ($\beta = 0.41$; $t = 3.42$; $p = 0.001$). In multivariate analysis, cirrhosis was associated with higher levels of IL-6 (OR = 15.27; 95% CI 1.28–182.50; $p = 0.03$), but not with IL-10, IL-17A and/or TNF-α ($p > 0.05$). Cirrhosis was also associated with alcohol abuse/dependence (OR = 3.11; 95% CI 1.10–9.67; $p = 0.04$).

Conclusions: In the current study, alcohol abuse was associated with an imbalance in IL-6 production in CHC patients. Moreover, the highest levels of IL-6 were found in patients with hepatic necroinflammatory activity graded as moderate/severe. PROEX, PRPq, CAPEs, CNPq.

P0744

PREVALENCE OF CO-MORBIDITIES BY HEPATITIS C VIRUS STATUS IN THE U.S. POPULATION

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Background and Aims: An estimated 2.2–3.0% people worldwide are affected by hepatitis C virus (HCV) infection. Some people infected with HCV may also have other chronic common co-morbid conditions such as chronic kidney disease (CKD), obesity and type 2 diabetes mellitus, which may affect how they respond to treatment for HCV. The prevalence of these chronic co-morbid conditions amongst people infected with HCV has not been well characterized and may vary by country. Therefore, the purpose of this study was to provide estimates of the prevalence of common co-morbid conditions among people infected with HCV in the U.S.

Methods: A cross-sectional analysis was done using data from a representative sample of the non institutionalized U.S. adult population which was collected as part of the 1999–2012 National Health and Nutrition Examination Surveys (NHANES). Data from survey participants aged ≥20 years who received a physical examination at the Mobile Examination Center were included in the study. As part of NHANES, serum samples were analyzed for the presence of anti-HCV using Ortho HCV enzyme-linked immunosorbent assay (ELISA), and confirmed using a recombinant immunoblot assay (RIBA) (RIBA HCV 3.0 Strip Immunoblot Assay). Age, sex, and race adjusted prevalence estimates of common co-morbid conditions were calculated amongst persons with or without antibodies to HCV.

Results: Overall the prevalence of anti-HCV in the U.S. was 1.8%. Higher anti-HCV prevalence was observed among those aged 40–50 years of age (3.6%), non-Hispanic blacks (3.3%), men (2.4%), and those living in poverty (3.7%). Age, sex and race adjusted estimates of the prevalence of co-morbidities by anti-HCV status are shown in the Table. Compared to those anti-HCV negative, adults with anti-HCV positive results had significantly higher prevalence of hypertension, history of heart failure, stroke, albuminuria, and low cholesterol levels.

Table: Age, sex and race adjusted prevalence (95% CI) of selected co-morbid conditions in the U.S. adult population (NHANES 1999–2012) by anti-hepatitis C virus status

	Anti-HCV positive	Anti-HCV negative	P value
Diabetes, %	10.9 (7.8–14.11)	10.2 (9.7–10.6)	0.62
Uncontrolled diabetes (HbA1c >7%)	46.7 (30.9–62.5)	48.5 (45.8–51.1)	0.830
Hypertension, %	36.5 (32.6–40.5)	32.3 (31.3–33.3)	0.03
Congestive heart failure, %	4.9 (2.6–7.2)	2.3 (2.1–2.5)	0.002
Stroke, %	5.5 (3.6–7.5)	2.7 (2.4–2.9)	<0.001
Dyslipidemias [a], %			
Low total cholesterol	41.6 (36.6–46.7)	31.5 (30.8–32.2)	<0.001
Low LDL-cholesterol	42.7 (34.1–51.4)	29.5 (28.4–30.5)	0.002
Low HDL-cholesterol	24.7 (17.2–32.2)	27.8 (25.8–29.7)	0.46
Low triglycerides	27.9 (21.4–34.3)	28.6 (27.4–29.9)	0.82
Albuminuria [b], %	14.5 (10.9–18.1)	10.9 (10.4–11.4)	0.034
eGFR <60 mL/min/1.73m^2, %	18.5 (14.5–22.5)	16.5 (15.7–17.4)	0.302
Obesity, %	23.1 (19.0–27.2)	33.3 (32.3–34.3)	<0.001
Metabolic syndrome, %	19.3 (15.2–23.5)	15.4 (14.5–16.3)	0.06
Anemia, %	5.8 (3.8–7.8)	7.4 (6.8–8.0)	0.17

[a] Lowest sex-specific quartile of each lipid parameter: Total cholesterol <177 mg/dL for men and <180 mg/dL for women; LDL-cholesterol: <100 mg/dL for men and <96 mg/dL for women; HDL-cholesterol: <38 mg/dL for men and <46 mg/dL for women ; triglycerides: <90 mg/dL for men and <79 mg/dL for women.
[b] Albuminuria >30 mg/g.

Conclusions: The study suggests that there may be a substantial burden of co-morbid conditions among individuals who previously or currently are infected with HCV (anti-HCV positive). Additional analysis will have to be done to look at the prevalence of these co-morbid conditions in people with chronic HCV since these are the patients that will be the target for the new treatment regimens that are being developed.

P0745

EQ-5D UTILITY INDEX IN FRENCH PATIENTS WITH CHRONIC HEPATITS C (CHC) INFECTION: SEVERE COMORBIDITIES AND PERCEIVED PROGRESSION OF CHC INFECTION MATTER MORE THAN ACTUAL LIVER DISEASE STAGE

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Background and Aims: Determinants of health-related quality-of-life and utilities associated with CHC infection are not well known.

Variables	N	EQ-5D	p
Gender			0.0383
Female	175	0.78	
Male	182	0.83	
Age			0.0213
18	93	0.82	
50	76	0.82	
55	85	0.83	
60	57	0.79	
65	46	0.71	
Place of birth			0.9861
France	257	0.80	
Other countries	100	0.80	
Education			<0.001
No bachelor level	162	0.75	
Bachelor level	195	0.85	
Centers			<0.001
Paris	188	0.85	
Lille	79	0.74	
Montpellier	90	0.76	
Genotype			0.1078
1	238	0.82	
2 and 3	66	0.76	
4, 5 and 6	53	0.80	
Fibrosis			0.0114
No severe	240	0.82	
Severe	117	0.76	
Treatment-naïve			0.3461
Naïve	174	0.81	
No naïve	183	0.79	
ALD no CHC			0.0003
ALD other disease	138	0.75	
No other disease	119	0.84	
Comorbidity			
No overweight	207	0.83	0.0082
Overweight	150	0.77	
No psychology disease	323	0.82	0.0012
Psychology disease	34	0.69	
No IDU	209	0.80	0.6419
IDU	148	0.81	
No alcohol	240	0.81	0.5061
Alcohol	117	0.79	
Perception evolution CHC		<0.001	
Very reassuring	39	0.85	
Rather reassuring	189	0.84	
Moderately disturbing	84	0.75	
Very worrying	23	0.61	

Methods: We conducted a cross-sectional study in three referral hepatology centers in Northern and Southern regions of France and in Paris. Patients aged 18–70 with CHC mono-infection were prospectively enrolled and interviewed before treatment initiation. The structured questionnaire included the EQ-5D-3L instrument. Patients perceptions on CHC infection and new antivirals was also assessed. In particular, patients were asked if they found that the CHC disease progression over the past was: very comforting, rather comforting, rather worrying, very worrying. Determinants of the EQ-5D utility index, and the overall health, as measured by a visual analogue scale (VAS), were analyzed in mixed models taking into account the clustering effect of hepatology centers.

Results: By Nov. 2014, 357 patients were enrolled. Patients' main characteristics were: 51% male; mean (SD) age of 54.0 (9.6) years old; 55% had completed high school; 42% were overweight (BMI >25); 39% had severe comorbidities other than liver disease stage; 67% had CHC genotype 1; 33% had severe liver fibrosis (F3–F4); 49% were treatment-naïve, and patient had never been treated with new direct-acting antivirals (DAA). Overall, 12%, 56%, 25%, and 7% patients perceived that CHC disease progression was very comforting, rather comforting, rather worrying, very worrying, respectively.

In the univariate analysis, low EQ-5D utility index was associated with female gender, older age, lower education attainment, severe liver fibrosis, having severe comorbidities, being overweight, having psychiatric diseases, and a bad perception of CHC progression (Table). In the multivariate analysis, when individual perceptions on CHC disease progression were introduced in the model, in contrast to patients' characteristics and comorbidities, liver fibrosis stage was no more associated with EQ-5D utility index. Similar results were found with use of the VAS.

Conclusions: This study suggests that comorbidities and perceptions of CHC disease progression highly impact patient's quality of life. It would be important to evaluate the impact of highly efficacious new direct acting agents on these variables that may not be associated with the underlying liver disease.

P0746

LOW VIRAEMIA IN HCV-INFECTED HAEMODIALYSED PATIENTS DEPENDS ON IL28B GENOTYPE

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Background and Aims: Low viraemia in HCV-infected haemodialysed patients have repeatedly been reported in the past. Two different hypotheses were postulated to explain this fact. The one hypothesis is a simple adsorption of the HCV virus on the haemodialysis membrane, the other hypothesis is based on an immune mediated decrease owing to higher levels of interferon alpha during haemodialysis session. The aim of or study was to support one of the mentioned hypotheses explaining low HCV RNA levels in haemodialysed patients.

Methods: The study compared pretreatment variables of 39 haemodialysed patients with 109 control patients with normal renal function. All patients in both groups were infected with HCV genotype 1b and were treated with pegylated interferon alpha and ribavirin. Viraemia below 600,000 IU/ml was considered as low.

Results: Pretreatment viraemia was significantly lower in haemodialysed patients than in controls (median HCV RNA 193,000 IU/ml, IQR 16,000–810,000 IU/ml; median 540,500 IU/ml, IQR 163,250–1,852,500 IU/ml, respectively, $p = 0.003$). IL28B CC

genotype carriers among haemodialysed patients had significantly lower viraemia, such a difference was not found in controls (see Figure). No other factors associated with low pretreatment viraemia in haemodialysed patients were found (age, sex, BMI, duration of haemodialysis). Haemodialysed patients with low pretreatment vireamia (10/11 CC genotype carriers and 14/28 non-CC genotype carriers) achieved significantly higher SVR rate ($p < 0.001$) than patients with high viraemia (1/11 and 0/10, respectively).

Conclusions: Low HCV vireamia in haemodialysed patients may be caused by a so far unknown immune mediated mechanism which is more pronounced in IL28B CC genotype carriers and facilitates interferon alpha induced clearance of the virus.

	Haemodialysed patients		Controls	
IL28B genotype	CC	Non-CC	CC	Non-CC
Number	11	28	21	88
HCV RNA [IU/ml]	12,400	323,500	905,000	531,000
median, IQR	1,750–66,300	40,850–1,680,000	12,950–2,350,000	167,000–1,890,000
	p = 0.003		p = NS	

P0747

HEALTH RELATED QUALITY OF LIFE AND UTILITY VALUES IN CHRONIC HEPATITIS C PATIENTS: A CROSS-SECTIONAL STUDY IN FRANCE, THE UK AND GERMANY

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Background and Aims: Accurate measuring of Health Related Quality of Life (HRQoL) is a major issue, especially in chronic disease as Chronic Hepatitis C (CHC). HRQoL could be derived as weights within quality-adjusted life year measurement (utility values), one of the frequently used outcomes in cost-effectiveness analysis. No utility values stratified by CHC health state have been retrieved in France. Recent data on HRQoL are also scarce. Two objectives of this study were to estimate HRQoL and utility values of CHC patients in 3 European countries.

Methods: This study is a cross-sectional one using data from the Adelphi Hepatitis C Disease Specific Programme implemented in France, the UK and Germany. In each country, 60 physicians recruited 10 consecutive CHC out-patients. They were asked to collect patients' demographic and clinical characteristics. Patients were invited to complete the EQ-5D questionnaire. This questionnaire is composed of two sections: (1) a description of the health state on 5 dimensions converted to a single value (called utility); (2) a Visual Analogue Scale (VAS) ranging from 0 to 100 (worst to best imaginable health state), known as a proxy of HRQoL. In order to improve accuracy of the utility values, clinically similar health states were merged. The French value set was used to derive utilities. For HRQoL, influence of patients' characteristics has been studied using univariate analyses and generalized least squares random effects model. Both utility values and VAS scores were presented by health state.

Results: 831 patients filled out the EQ-5D questionnaire. The mean age was 47.1±14.1 years old; 58.8% of them were male. French patients were slightly older; a higher proportion of patients infected by drug injection was found in the UK. As expected, as liver disease progressed, utility values decreased (Table). Increase in utility values was higher in patients with a Sustained Virologic Response (SVR) from F2–F3–F4 fibrosis stage (+27%). For HRQoL, results from multivariate analysis showed that 4 variables had significant negative impact: liver disease stage, number of concomitant conditions, being an intravenous drug user and being unemployed. Positive response to treatment and having some knowledge about the disease had a positive impact on HRQoL.

Table. VAS and utility mean scores stratified by health state

Health state (N = 831)	VAS, Mean (SE)	Utility, Mean (SE)
F0–F1 (N = 239)	72.0 (1.08)	0.82 (0.02)
F2 (N = 246)	65.2 (1.10)	0.78 (0.02)
F3–F4 (N = 101)	55.9 (1.98)	0.67 (0.03)
Decompensated cirrhosis / Hepatocellular carcinoma (N = 25)	55.4 (3.73)	0.51 (0.07)
Liver transplant		
initial year (N = 5)	52.0 (3.74)	0.46 (0.10)
subsequent years (N = 10)	73.0 (6.48)	0.80 (0.08)
Unclassified (N = 205)	67.5 (1.40)	0.79 (0.02)
SVR		
from F0–F1 (N = 35)	80.2 (2.46)	0.95 (0.01)
from F2–F3–F4 (N = 36)	66.1 (2.68)	0.85 (0.02)

Conclusions: This utility value set might be helpful to populate cost-effectiveness analysis. Consideration of EQ-5D and CHC-specific HRQoL questionnaires in large national studies will be useful for confirming these findings.

P0748

COURSE OF HCV RNA AND HCV CORE ANTIGEN TESTING ARE PREDICTORS FOR REACHING SVR IN TRANSPLANT RECIPIENTS

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Background and Aims: Hepatitis C in liver transplant recipients (LTR) is associated with reduced graft survival. The former standard therapy consisting of peg interferon and ribavirin was associated with the risk of rejection and with relevant side effects. The new direct acting antivirals (DAAs) seem to be a safe and efficient therapeutic option, but the knowledge on the use of these cost intensive drugs in LTR is limited. Thus a predictor of sustained virological response (SVR) is needed to identify LTR who benefit from this expensive therapy. The HCV antigen assay (HCV Ag) has shown to be a cheap and efficient method to detect HCV but the value of this technique to predict SVR in LTR still needs to be determined.

Methods: All LTR who have been treated with Sofosbuvir at our center between 03/2014 and 08/2014 were analysed (n = 20). HCV Ag and HCV RNA (PCR) were determined each visit (week 1, 2, 4, 8, 12 and 24 of treatment). Primary endpoints of this study were SVR 4 and SVR 12.

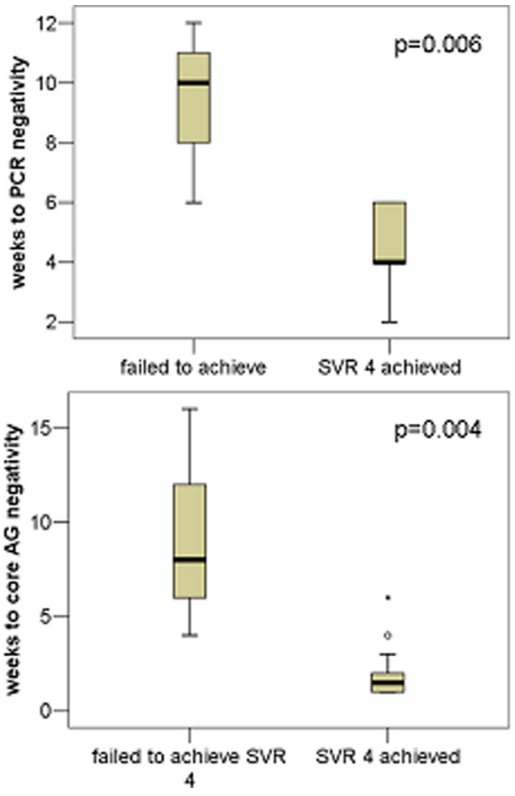
Results: To date 20 patients have been treated. The majority was male (n = 14, 70%). The age ranged from 31–68 years (mean 56). Mean baseline HCV RNA was 3506500 IU/mL [range 5000–10,000,000 IU/mL]. Patients were infected with HCV genotype (GT) 1 (n = 12), GT 2 (n = 3), GT 3 (n = 1), GT 1 and 3 (n = 1) and GT 4 (n = 3). Patient's immunosuppresion contained CSA (n = 3), CSA and MMF (n = 6), CSA and Pred (n = 1), Tac (n = 3), Tac and EVR (n = 3), Tac and MMF (n = 1) and Tac and Pred (n = 1). DAA medication consisted of sofosbuvir plus ribavirin (n = 14), sofosbuvir, simeprevir and ribavirin (n = 5) and sofosbuvir, peg interferon and ribavirin (n = 1).

HCV core antigen tested negative after a mean of 3 weeks (range 1–16) and PCR tested negative after a mean of 5 weeks (range 2–12).

Currently 18 patients have reached week 4 after end of treatment and 15/18 (83%) achieved SVR 4. A short interval between treatment start and HCV PCR negativity ($p = 0.006$) or HCV Ag negativity ($p = 0.004$) was associated with reaching SVR 4 (figure). No severe side effects were observed.

Conclusions: DAA treatment is safe and well tolerated in LTR. The time point of HCV Ag loss and PCR negativity was a predictor of SVR 4. In this pilot study the HCV Ag seems to be superior to predict

SVR in comparison to PCR (p = 0.004 vs p = 0.006). However we can currently not draw further valid conclusions as SVR 12 results are pending.



P0749

IMPLICATION OF TYPE 2 DIABETES SUSCEPTIBILITY LOCI IN HEPATITIS C VIRUS INFECTION

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Background and Aims: There is a significant association between hepatitis C virus (HCV) infection and the risk of type 2 diabetes (T2D). However, the implications of genetic susceptibility loci for T2D in populations with HCV infection are unclear. We therefore conducted a study to detect susceptibility loci for T2D in Chinese infected with HCV.

Methods: A total of 100 representative single-nucleotide polymorphisms (SNPs) in susceptibility loci for T2D that were previously reported in GWAS were genotyped in 762 Han Chinese with HCV infection by using illumina infinium iSelect HD Custom Genotyping BeadChips.

Table 1. Baseline of patients infected with HCV.

	Spontaneous clearers (N = 155)	HCV persisters (N = 607)
Age (years), mean±SD	51.88±7.44	48.84±10.16
Gender (male/female)	76/72	334/231
HCV genotype (1b/others)	NR	217/107
HCV RNA levels (high/low)	NR	494/107

Data are presented as mean± standard deviation.
HCV RNA levels: high, ≥5 log IU/mL; low, <5 log IU/mL.

Results: 607 patients infected with HCV (persisters) and 155 spontaneous resolvers were enrolled in this study. Baseline characteristics of the patients are shown in Table 1. Table 2 summarizes the results of these SNPs. Sixteen genetic variants (rs340874, rs243021, rs6712932, rs780094, rs4689388, rs12518099, rs864745, rs3802177, rs896854, rs564398, rs649891, rs1111875, rs1552224, rs5219a, rs1359790, and rs7119) were not identified, and 14 SNPs with minor allele frequency (MAF) < 0.05 (rs10923931, rs7578597, rs11708067, rs10010131, rs1801214, rs7636, rs12255372, rs4506565, rs7901695, rs7903146, rs1153188, rs7957197, rs8042680, and rs11642841) were excluded in the following analyses. In comparison with data of Han Chinese from HapMap (Table 3), the higher risk allele frequencies of 21 SNPs (rs11677370, rs7593730, rs4607103, rs10440833, rs4712523, rs6931514, rs7756992, rs9465871, rs4607517, rs972283, rs13266634, rs10741243, rs12779790, rs2237892, rs5215, rs1495377, rs730570, rs11634397, rs7172432, rs472265, and rs2833610) were reported in HCV persistence and spontaneous resolvers. There were no significant differences between HCV persistence and spontaneous resolvers (P < 0.05). Among them, the risk allele frequency of rs1495377 mapped in *TSPAN8-LGR5* was three times higher than that in general population in China. The risk allele frequency of rs7020996 in HCV persistence was higher than that in spontaneous resolvers (P < 0.05). On the contrary, the risk allele frequencies of rs2063640, rs5015480 and rs6583826 in spontaneous resolvers were higher (P < 0.05).

Table 2 (abstract P0749). Risk allele frequencies of SNPs in susceptibility loci for T2D in HCV persisters and spontaneous resolvers.

No.	Chr	SNP	Reported gene(s)	Mapped gene	RefSNP Alleles	Risk allele				
						Allele	Frequency in HCV persisters	Frequency in spontaneous resolvers	P	OR
1	1	rs10923931	NOTCH2, ADAM30	NOTCH2	G/T	T	0.03215	0.032	1	1.005
2	1	rs12027542	*PCNXL2*	*PCNXL2;LOC101927736*	A/G	G	0.2725	0.3	0.3925	0.874
3	1	rs17045328	*CR2*	*CR2*	A/G	G	0.3105	0.32	0.7643	0.9569
4	1	rs340874	*PROX1-AS1*	*PROX1*	A/G	C	N/A	N/A		
5	2	rs11677370	*Intergenic*	*DCDC2C*	A/T	A	0.3978	0.4195	0.5588	0.9142
6	2	rs243021	*BCL11A*	*RNA5SP94 – MIR4432*	C/T	A	N/A	N/A		
7	2	rs2943641	*LOC64673, IRS1*	*NYAP2 – MIR5702*	C/T	C	0.92978	0.9	0.1135	0.6797
8	2	rs6712932	*Intergenic*	*MRPS9 – GPR45*	C/T	G	N/A	N/A		
9	2	rs7578326	*IRS1*	*LOC646736*	A/G	A	0.8458	0.82	0.3405	0.8308
10	2	rs7578597	*THADA*	*THADA*	C/T	T	0.98985	0.988	0.7349	0.8444
11	2	rs7593730	*RBMS1, ITGB6*	*RBMS1*	C/T	C	0.8291	0.816	0.6456	0.9141
12	2	rs780094	*GCKR*	*GCKR*	A/G	T	N/A	N/A		
13	3	rs11708067	*ADCY5*	*ADCY5*	A/G	A	0.994924	1	0.5978	0
14	3	rs13081389	*PPARG*	*TIMP4 – GSTM5P1*	A/G	A	0.92942	0.944	0.4894	1.28
15	3	rs1470579	*IGF2BP2*	*IGF2BP2*	A/C	C	0.2637	0.22	0.1746	1.269
16	3	rs17036101	*SYN2, PPARG*	*TIMP4 – GSTM5P1*	A/G	G	0.92809	0.944	0.4128	1.306

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Table 2, *continued* (abstract P0749). Risk allele frequencies of SNPs in susceptibility loci for T2D in HCV persisters and spontaneous resolvers.

No.	Chr	SNP	Reported gene(s)	Mapped gene	RefSNP Alleles	Risk allele				
						Allele	Frequency in HCV persisters	Frequency in spontaneous resolvers	P	OR
17	3	rs1801282	*PPARG*	*PPARG*	C/G	C	0.91201	0.932	0.3804	1.322
18	3	rs2063640	*ZPLD1*	*ZPLD1 – NDUFA4P2*	A/C	A	0.2428	0.308	0.0374	0.7204
19	3	rs358806	*NR*	*LRTM1 – WNT5A*	A/C	A	0.187	0.168	0.5296	1.139
20	3	rs4402960	*IGF2BP2*	*IGF2BP2*	G/T	T	0.2513	0.208	0.168	1.278
21	3	rs4607103	*ADAMTS9*	*ADAMTS9-AS2*	C/T	C	0.6063	0.616	0.8306	1.042
22	3	rs6769511	*IGF2BP2*	*IGF2BP2*	C/T	C	0.2632	0.22	0.1746	1.267
23	3	rs7630877	*PEX5L*	*PEX5L*	A/G	A	0.1557	0.136	0.4976	1.171
24	4	rs10010131	*WFS1*	*WFS1*	A/G	G	0.95734	0.964	0.729	1.193
25	4	rs1801214	*WFS1*	*WFS1*	A/C/G/T	T	0.96102	0.964	1	1.086
26	4	rs3792615	*MARCH1*	*MARCH1*	A/G	G	0.1797	0.156	0.4106	1.185
27	4	rs4689388	*WFS1, PPP2R2C*	*WFS1*	A/G	T	N/A	N/A		
28	4	rs7659604	*NR*	*ANXA5 – TMEM155*	C/T	T	0.3829	0.416	0.3532	0.8709
29	5	rs12518099	*LOC72901, CETN3*	*LOC101929495*	A/G	C	N/A	N/A		
30	6	rs10440833	*CDKAL1*	*CDKAL1*	A/T	A	0.4137	0.424	0.7778	0.9586
31	6	rs1048886	*C6orf57*	*C6orf57*	A/G	G	0.06803	0.092	0.1803	0.7204
32	6	rs10946398	*CDKAL*	*CDKAL1*	A/C	C	0.439	0.416	0.5278	1.098
33	6	rs4712523	*CDKAL1*	*CDKAL1*	A/G	G	0.4406	0.432	0.8335	1.035
34	6	rs4712524	*CDKAL1*	*CDKAL1*	A/G	G	0.4407	0.4194	0.5734	1.091
35	6	rs642858	*Intergenic*	*ATP5F1P6 – RNA5SP220*	C/T	T	0.3569	0.4344	0.0239	0.7225
36	6	rs6931514	*CDKAL1*	*CDKAL1*	A/G	G	0.5144	0.524	0.8345	1.039
37	6	rs7754840	*CDKAL1*	*CDKAL1*	C/G	C	0.4371	0.416	0.5742	1.09
38	6	rs7756992	*CDKAL1*	*CDKAL1*	A/G	G	0.5161	0.52	0.9445	1.016
39	6	rs9465871	*CDKAL1*	*CDKAL1*	C/T	C	0.5162	0.52	0.9445	1.015
40	6	rs9472138	*VEGFA*	*TRNAI25*	C/T	T	0.1032	0.136	0.1458	0.7312
41	7	rs2191349	*NR*	*DGKB*	G/T	T	0.6331	0.62	0.7185	0.9457
42	7	rs4607517	*NR*	*GCK*	A/G	A	0.2047	0.208	0.9313	0.9803
43	7	rs7636	*ACHE*	*ACHE*	C/T	T	0.00423	0	0.594	NA
44	7	rs849134	*JAZF1*	*JAZF1*	A/G	A	0.7547	0.792	0.2213	1.238
45	7	rs864745	*JAZF1*	*JAZF1*	A/G	T	N/A	N/A		
46	7	rs972283	*KLF14*	*KLF14 – MIR29A*	A/G	G	0.7042	0.72	0.6469	1.08
47	8	rs13266634	*SLC30A8*	*SLC30A8*	C/T	C	0.5995	0.588	0.7764	0.9535
48	8	rs3802177	*SLC30A8*	*SLC30A8*	C/T	G	N/A	N/A		
49	8	rs896854	*TP53INP1*	*TP53INP1;LOC101927002*	A/G	T	N/A	N/A		
50	9	rs10811661	*CDKN2A, CDKN2B*	*UBA52P6 – DMRTA1*	C/T	T	0.54	0.46	0.0255	0.7258
51	9	rs10965250	*CDKN2A,CDKN2B*	*UBA52P6 – DMRTA1*	A/G	G	0.5567	0.46	0.0063	0.6783
52	9	rs1333051	*CDKN2A, CDKN2B*	*UBA52P6 – DMRTA1*	A/T	A	0.8393	0.804	0.191	0.7857
53	9	rs17584499	*PTPRD*	*PTPRD*	C/T	T	0.08629	0.06	0.2033	1.48
54	9	rs2383208	*CDKN2A*	*UBA52P6 – DMRTA1*	A/G	A	0.5671	0.48	0.0142	0.7047
55	9	rs564398	*CDKN2A, CDKN2B*	*CDKN2B-AS1*	A/G	C	N/A	N/A		
56	9	rs649891	*PTPRD*	*PTPRD*	A/G	C	N/A	N/A		
57	9	rs7020996	*CDKN2A,CDKN2B*	*UBA52P6 – DMRTA1*	C/T	C	0.5764	0.504	0.0416	0.7468
58	10	rs10741243	*TCERG1L*	*TCERG1L*	C/G	C	0.1162	0.124	0.7456	0.9292
59	10	rs10906115	*CDC123,CAMK1D*	*CDC123 – CAMK1D*	A/G	A	0.6043	0.6492	0.1971	1.212
60	10	rs1111875	*HHEX*	*HHEX – EXOC6*	A/G	C	N/A	N/A		
61	10	rs12255372	*TCF7L2*	*TCF7L2*	G/T	G	0.98308	0.992	0.4037	2.134
62	10	rs12779790	*CDC123,CAMK1D*	*CDC123 – CAMK1D*	A/G	G	0.1434	0.1748	0.2358	0.79
63	10	rs4506565	*TCF7L2*	*TCF7L2*	A/T	T	0.05161	0.036	0.3371	1.457
64	10	rs5015480	*HHEX*	*HHEX – EXOC6*	C/T	C	0.1851	0.252	0.018	0.674
65	10	rs6583826	*KIF11*	*IDE – KIF11*	A/G	G	0.236	0.3	0.0359	0.7207
66	10	rs7901695	*TCF7L2*	*TCF7L2*	C/T	C	0.05169	0.036	0.3369	1.46
67	10	rs7903146	*TCF7L2*	*TCF7L2*	C/T	T	0.05161	0.036	0.3371	1.457
68	11	rs10830963	*MTNR1B*	*MTNR1B*	C/G	G	0.4144	0.3911	0.5232	1.102
69	11	rs1387153	*MTNR1B*	*RPS3AP42 – MTNR1B*	C/T	T	0.3839	0.3911	0.8299	0.97
70	11	rs1552224	*CENTD2*	*ARAP1*	G/T	A	N/A	N/A		
71	11	rs163182	*KCNQ1*	*KCNQ1*	C/G	C	0.3486	0.356	0.827	0.9679
72	11	rs2237892	*KCNQ1*	*KCNQ1*	C/T	C	0.6958	0.708	0.7617	1.06
73	11	rs2237895	*KCNQ1*	*KCNQ1*	A/C	C	0.3328	0.328	0.9411	1.022
74	11	rs2237897	*KCNQ1*	*KCNQ1*	C/T	C	0.676	0.664	0.711	0.9473
75	11	rs5215	*KCNJ11*	*KCNJ11*	C/T	C	0.4148	0.4919	0.0285	0.7321
77	11	rs5219a	*KCNJ11*	*KCNJ11*	C/T	?	N/A	N/A		
78	11	rs9300039	*Intergenic*	*RPL9P23 – HNRNPKP3*	A/C	C	0.7262	0.712	0.6411	0.9321
79	12	rs1153188	*DCD*	*DCD – VDAC1P5*	A/T	A	0.01523	0.016	1	0.951
80	12	rs12304921	*NR*	*HIGD1C*	A/G	G	0.5136	0.484	0.4041	0.8884
81	12	rs1495377	*NR*	*TSPAN8 – LGR5*	C/G	G	0.7602	0.748	0.6849	0.9365
82	12	rs1531343	*HMGA2*	*RPSAP52*	C/G	C	0.128	0.1169	0.6752	1.108
83	12	rs4760790	*TSPAN8,LGR5*	*TSPAN8 – LGR5*	A/G	A	0.2042	0.248	0.1258	0.7782
84	12	rs7305618	*HNF1A*	*RPL12P33 – HNF1A-AS1*	C/T	C	0.5214	0.456	0.0699	0.7695

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Table 2, *continued* (abstract P0749). Risk allele frequencies of SNPs in susceptibility loci for T2D in HCV persisters and spontaneous resolvers.

No.	Chr	SNP	Reported gene(s)	Mapped gene	RefSNP Alleles	Risk allele				
						Allele	Frequency in HCV persisters	Frequency in spontaneous resolvers	P	OR
85	12	rs7957197	*OASL*	*OASL*	A/T	T	0.999154	1	1	0
86	12	rs7961581	*TSPAN8,LGR5*	*TSPAN8 – LGR5*	C/T	C	0.1797	0.208	0.3234	0.8339
87	13	rs1359790	*SPRY2*	*LINC01080 – SPRY2*	C/T	G	N/A	N/A		
88	14	rs730570	*C14orf70*	*LINC00523 – DLK1*	A/G	G	0.8528	0.836	0.4959	0.8799
89	15	rs11634397	*ZFAND6*	*ZFAND6 – FAH*	A/G	G	0.11	0.116	0.8246	0.9417
90	15	rs1436955	*C2CD4B*	*NPM1P47 – C2CD4B*	A/G	A	0.2521	0.24	0.7479	1.067
91	15	rs7119	*HMG20A*	*HMG20A*	A/G	T	N/A	N/A		
92	15	rs7172432	*C2CD4A, C2CD4B*	*NPM1P47 – C2CD4B*	A/G	A	0.5915	0.608	0.6707	1.071
93	15	rs8042680	*PRC1*	*PRC1;PRC1-AS1*	A/C	A	0.990694	0.996	0.7035	2.339
94	16	rs11642841	*FTO*	*FTO*	A/C	A	0.03976	0.032	0.7171	1.253
95	16	rs8050136	*FTO*	*FTO*	A/C	A	0.1252	0.112	0.5979	1.135
96	16	rs9939609	*FTO*	*FTO*	A/T	A	0.1261	0.112	0.5976	1.144
97	17	rs391300	*SRR*	*SRR*	A/G	G	0.7419	0.78	0.2288	1.233
98	18	rs10460009	*LPIN2*	*LPIN2;LOC727896*	C/T	T	0.4907	0.48	0.7808	1.044
99	19	rs472265	*FLJ16165*	*PAPL*	A/G	G	0.3175	0.304	0.7082	1.065
100	21	rs2833610	*HUNK*	*HUNK – LINC00159*	A/G	G	0.4634	0.488	0.4859	0.906
101	X	rs5945326	*DUSP9*	*KRT18P48 – DUSP9*	A/G	G	0.3313	0.3153	0.693	1.076

MAF, minor allele frequency; N/A, not available.

Table 3 (abstract P0749). Data from Hapmap and results for risk allele frequencies of SNPs in susceptibility loci for T2D

No.	Chr	SNP	Risk allele	Minor allele	*RAF in CHB	Risk allele frequency	
						In HCV persisters	In spontaneous resolvers
2	1	rs12027542	G	G	0.298	0.2725	0.3
3	1	rs17045328	G	G	0.381	0.3105	0.32
5	2	rs11677370	A	A	0.289	0.3978	0.4195
7	2	rs2943641	C	T	0.929	0.92978	0.9
9	2	rs7578326	A	G	0.863	0.8458	0.82
11	2	rs7593730	C	T	0.798	0.8291	0.816
14	3	rs13081389	A	G	0.978	0.92942	0.944
15	3	rs1470579	C	C	0.262	0.2637	0.22
16	3	rs17036101	G	A	0.978	0.92809	0.944
17	3	rs1801282	C	G	0.952	0.91201	0.932
18	3	rs2063640	A	A	0.274	0.2428	0.308
19	3	rs358806	A	A	0.19	0.187	0.168
20	3	rs4402960	T	T	0.238	0.2513	0.208
21	3	rs4607103	C	T	0.577	0.6063	0.616
22	3	rs6769511	C	C	0.262	0.2632	0.22
23	3	rs7630877	A	A	0.19	0.1557	0.136
26	4	rs3792615	G	G	N/A	0.1797	0.156
28	4	rs7659604	T	C	0.607	0.3829	0.416
30	6	rs10440833	A	A	0.389	0.4137	0.424
31	6	rs1048886	G	G	0.107	0.06803	0.092
32	6	rs10946398	C	C	0.423	0.439	0.416
33	6	rs4712523	G	G	0.423	0.4406	0.432
34	6	rs4712524	G	N/A	N/A	0.4407	0.4194
35	6	rs642858	T	N/A	N/A	0.3569	0.4344
36	6	rs6931514	G	G	0.456	0.5144	0.524
37	6	rs7754840	C	C	0.423	0.4371	0.416
38	6	rs7756992	G	G	0.476	0.5161	0.52
39	6	rs9465871	C	C	0.5	0.5162	0.52
40	6	rs9472138	T	T	0.125	0.1032	0.136
41	7	rs2191349	T	G	0.711	0.6331	0.62
42	7	rs4607517	A	A	0.19	0.2047	0.208
44	7	rs849134	A	G	0.814	0.7547	0.792
46	7	rs972283	G	A	0.667	0.7042	0.72
47	8	rs13266634	C	T	0.53	0.5995	0.588
50	9	rs10811661	T	C	0.577	0.54	0.46
51	9	rs10965250	G	A	0.581	0.5567	0.46
52	9	rs1333051	A	T	0.839	0.8393	0.804
53	9	rs17584499	T	T	0.089	0.08629	0.06
54	9	rs2383208	A	G	0.605	0.5671	0.48
57	9	rs7020996	C	T	0.567	0.5764	0.504
58	10	rs10741243	C	C	0.111	0.1162	0.124
59	10	rs10906115	A	G	0.649	0.6043	0.6492

Continued on next page.

Table 3, *continued* (abstract P0749). Data from Hapmap and results for risk allele frequencies of SNPs in susceptibility loci for T2D

No.	Chr	SNP	Risk allele	Minor allele	*RAF in CHB	Risk allele frequency	
						In HCV persisters	In spontaneous resolvers
62	10	rs12779790	G	G	0.133	0.1434	0.1748
64	10	rs5015480	C	C	0.202	0.1851	0.252
65	10	rs6583826	G	G	0.262	0.236	0.3
68	11	rs10830963	G	G	0.417	0.4144	0.3911
69	11	rs1387153	T	T	0.393	0.3839	0.3911
71	11	rs163182	C	C	0.367	0.3486	0.356
72	11	rs2237892	C	T	0.655	0.6958	0.708
73	11	rs2237895	C	N/A	N/A	0.3328	0.328
74	11	rs2237897	C	T	N/A	0.676	0.664
75	11	rs5215	C	C	0.345	0.4148	0.4919
78	11	rs9300039	C	A	0.714	0.7262	0.712
80	12	rs12304921	G	G	0.494	0.5136	0.484
81	12	rs1495377	G	G	0.274	0.7602	0.748
82	12	rs1531343	C	C	0.113	0.128	0.1169
83	12	rs4760790	A	A	0.244	0.2042	0.248
84	12	rs7305618	C	C	0.47	0.5214	0.456
86	12	rs7961581	C	C	0.196	0.1797	0.208
88	14	rs730570	G	A	0.792	0.8528	0.836
89	15	rs11634397	G	G	0.071	0.11	0.116
90	15	rs1436955	A	T	0.756	0.2521	0.24
92	15	rs7172432	A	G	0.56	0.5915	0.608
95	16	rs8050136	A	A	0.143	0.1252	0.112
96	16	rs9939609	A	A	0.149	0.1261	0.112
97	17	rs391300	G	T	N/A	0.7419	0.78
98	18	rs10460009	T	T	0.512	0.4907	0.48
99	19	rs472265	G	G	0.238	0.3175	0.304
100	21	rs2833610	G	G	0.458	0.4634	0.488
101	X	rs5945326	G	G	0.373	0.3313	0.3153

Conclusions: This is the first study performed to examine the prevalence of risk allele in susceptibility loci for T2D in Chinese with HCV infection. Our results suggest that 21 SNPs with higher risk allele frequencies should be explored.

P0750

ENHANCED LIVER FIBROSIS (ELF) TEST DOES NOT PREDICT RESIDUAL CIRRHOSIS AFTER A SUSTAINED VIROLOGICAL RESPONSE TO HEPATITIS C TREATMENT

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Background and Aims: Regression of cirrhosis is a potential clinical benefit of successful interferon alfa (IFN) therapy of hepatitis C virus (HCV) infection. Non invasive serum assays and transient elastography (TE) have suboptimal accuracy for diagnosis of cirrhosis in patients who achieved a cure of infection (SVR), yet the diagnostic accuracy of serum assays based upon direct markers of liver fibrosis is unknown. The aim is to investigate the accuracy of Enhanced Liver Fibrosis (ELF) test in identifying residual cirrhosis in SVR patients.
Methods: HCV patients with histologically diagnosed cirrhosis and a post-SVR liver biopsy (LB) were investigated with ELF test at baseline and after an SVR. Four cut-offs were used for the diagnosis of cirrhosis i.e. 9.5, 9.8, 10.3 and 11.3 and compared to histological staging of fibrosis by METAVIR. ELF performance was calculated using receiver operating characteristic (ROC) curves analysis.
Results: 19/29 patients (66%) had cirrhosis regression after a median of 61 (48–104) months from an SVR. Overall, median ELF values decreased from 10.7 to 8.6 at the time of SVR ($p < 0.0001$), independently from cirrhosis regression ($p < 0.0001$ in regressed and $p = 0.001$ in non-regressed patients). None of the four cut-offs showed an acceptable diagnostic accuracy for the identification of residual cirrhosis, as 4%, 3%, 0% and 0% of the patients showed the 9.5, 9.8, 10.3 and 11.3 cut-offs. By ROC analysis, the derived 8.1 cut-off was the most accurate however with a suboptimal performance (Sn 50%, Sp 68%, LR+ 1.58, LR− 0.73, AUROC 0.54). In SVR patients, ELF test did not show any correlation with fibrosis stage ($r = 0.34$), area of fibrosis ($r = 0.29$), or histological grading ($r = 0.35$) which did correlate with ELF test at baseline ($p = 0.02$).
Conclusions: ELF test, a direct marker of liver fibrosis, does not accurately identify residual cirrhosis in HCV patients who achieved an SVR, thus LB remains the reference standard for this endpoint.

P0751

POLYMORPHISMS IN THE FAS PROMOTER REGION ARE NOT ASSOCIATED WITH FIBROSIS PROGRESSION IN HCV-1 AND -4 PATIENTS

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Background and Aims: Identification of patients with chronic hepatitis C virus (HCV) infection and rapid fibrosis progression has significant prognostic and therapeutic implications. The rs1800682 A>G genetic polymorphism in the promoter region of the FAS gene, which is involved in liver cells apoptosis, has been associated with histological activity and lesser stages of fibrosis in HCV-4 Egyptian patients carrying the A allele. Its impact in HCV-1 patients as well as in HCV-4 patients of European descent is unknown.

Methods: We analyzed data from 2 published patients cohorts of HCV-1 and 4 patients with at least one pre-treatment liver biopsy. Liver staging and grading were evaluated using the Ishak Score. In patients with known date of infection, the fibrosis progression rate (FPR) was calculated as the ratio between biopsy staging score and years of disease. Genotype was assessed by using TaqMan SNP Genotyping Assay.
Results: Overall, 203 HCV infected patients (121 HCV-1, 82 HCV-4 of them 61% Egyptians) were analyzed. The date of infection could be estimated in 131 of them. No differences in rs1800682 distribution was observed between Italian and Egyptian patients: AA/AG 106/152 (69%), GG 46/152 (31%) *vs* AA/AG 34/50 (68%), GG 16/50 (32%). No differences in median FPR values were observed in subjects carrying the AA/AG or the GG genotypes (0.12 *vs* 0.13). In genotype 4 patients the different rs1800682 genotypes were equally distributed in patients with mild (S0–S1) fibrosis (AA/AG vs. GG: 72% vs 28%) and advanced (S3–S6) fibrosis (AA/AG vs. GG: 74% vs. 26%), p = 1.0. In HCV-1 patients we observed a protective role of A allele (AA/AG) in genotype 1 patients with mild fibrosis vs. advanced fibrosis: 52/70 (72%) vs. 28/51 (54%), p = 0.033. No impact of the rs1800682 polymorphism was seen on histological activity both overall and stratified by HCV genotype: Grading ≥7: AA/AG 54/83 (65%), GG 29/83 (35%); Grading <7: AA/AG 86/120 (71%), GG 34/120 (29%), p = 0.3.
Conclusions: The rs1800682 polymorphism is not a useful genetic predictor of liver fibrosis progression when assessed through FPR, fibrosis stage or inflammation grade in HCV genotype 1 and 4 patients.

P0752
NOTCH4 AND MHC CLASS II POLYMORPHISMS CONTIBUTE TO HCV-RELATED BENIGN AND MALIGNANT LYMPHOPROLIFERATIVE DISEASES

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Background and Aims: Hepatitis C virus (HCV) leads to chronic liver disease, but more rarely it causes lymphoproliferative disorders (LPDs). The most frequent HCV-related LPD is mixed cryoglobulinemia (MC). MC is clinically benign, but it evolves in about 10% of cases into a non Hodgkin's lymphoma (NHL). The exact pathogenesis of HCV-related LPDs is still unknown and few and conflicting data exists about the host genetic contribution. Recently, a GWAS study allowed the discovery of an association of two particular SNPs on chromosome 6 and HCV-related MC vasculitis; the first one is a SNP (rs2071286) located in an intronic region of the NOTCH4 gene, the second one is a SNP (rs9461776) located between HLA-DRB1 and HLA-DQA1 gene segments.
Aim: To define the contribution of rs2071286 and rs9461776 in the predisposition to develop HCV-related LPDs (MC and NHL).
Methods: rs2071286 and rs9461776 SNP were determined using TaqMan SNP Genotyping Assay in 438 patients: 85 HCV patients without LPDs (HCV), 73 HCV patients with circulating cryoglobulins (MC-HCV), 108 with HCV-associated MC syndrome (MCS-HCV), 62 with HCV-related NHL (NHL-HCV) and 110 HCV negative patients with NHL (NHL).
Results: Concerning rs2071286, significantly higher minor allele frequency (maf) was observed in all the case groups respect to controls (MC-HCV, $p = 0.035$; MCS-HCV, $p < 0.001$; NHL-HCV, $p = 0.001$ and NHL, $p = 0.019$). Comparing to HCV group, the odds-ratio associated with rs2071286 maf were: MC-HCV, OR 1.79; MCS-HCV, OR 2.59; NHL-HCV, OR 2.6; NHL, OR 1.81.
Regarding rs9461776, the higher maf was observed in NHL-HCV and in NHL groups ($p = 0.011$ and $p = 0.0283$ respect to HCV, respectively). Furthermore, we described an increasing trend, although not significant, following the evolution of the LPDs. The presence of minor allele of rs9461776 conferred an OR of 2.42 to NHL-HCV and an OR of 1.7 to NHL group, in respect to controls.
Conclusions: We confirm the previously demonstrated association between the two SNPs and HCV-related MC vasculitis (Zignego et al, 2014), and showed a similar associations for HCV-related NHLs and, regarding NOTCH4 SNP, also for patients with CGs but without symptoms. Furthermore, HCV negative NHLs showed higher frequencies of the two minor alleles respect to controls, but lower compared to HCV positive lymphomas. This suggests that HCV acts on a permissive genetic substrate and confirms the virus contribution to the pathogenesis of lymphoma.

P0753
GENETIC POLYMORPHISMS OF IL28B AND PNPLA3 ARE CLOSELY ASSOCIATED WITH FIBROSIS PROGRESSION IN CHRONIC HEPATITIS C

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Background and Aims: The assessment of individual risk of fibrosis progression in patients with chronic hepatitis C who failed interferon based antiviral therapy is an unmet clinical need.
Methods: Genetic risk factors associated with fibrosis progression were analyzed in 176 viremic patients who did not achieve sustained virological response by interferon-based therapy and linked to the fibrosis progression rate (FPR). FPR was determined in all patients by paired liver biopsy performed before and after therapy (mean interval: 6.2 years).
Results: Mean FPR in patients with IL28B (rs8099917) TG/GG and PNPLA3 (rs738409) CG/GG were significantly higher than in those with IL28B TT (FPR: 0.144 vs. 0.034, p < 0.001) and PNPLA3 CC (FPR: 0.10 vs. 0.018, p = 0.005), respectively. IL28B TG/GG [hazard ratio (HR): 2.1, p = 0.003] and PNPLA3 CG/GG (HR: 2.5, p = 0.004) remained independent predictors of fibrosis progression upon multivariate analysis together with age ≥60 years (HR: 2.2, $p = 0.003$), and average alanine aminotransferase after interferon therapy ≥40 IU/l (HR: 2.2, $P = 0.005$). Based on these data, we developed a new clinical score predicting the risk of fibrosis progression. This score identified subgroups of patients with a low (FPR: 0.00, HR: 1), intermediate (FPR: 0.06, HR: 3.0, $p = 0.007$), and high (FPR: 0.17, HR: 7.5, $p < 0.001$) risk of fibrosis progression. Further, cumulative incidence of hepatocellular carcinoma development was significantly higher in high risk patients than other patients ($P = 0.006$).
Conclusions: *IL28B/PNPLA3* genotypes are associated with rapid fibrosis progression.

P0754
CLINICAL SIGNIFICANCE OF DRUG–DRUG INTERACTIONS DURING THERAPY WITH NOVEL DAAS AGAINST HCV

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Background and Aims: Up to 50% of hepatitis C virus (HCV) infected patients (pts) are at risk for clinically significant drug–drug interactions (DDI) between first wave protease inhibitors (boceprevir/telaprevir) and their regular outpatient medication. This risk is considered to be lower with the second wave direct-acting antiviral agents (DAA) sofosbuvir (SOF), simeprevir (SMV), daclatasvir (DCV) and ledipasvir/sofosbuvir (LDV/SOF). We here aimed to evaluate the risk for DDI between the regular outpatient medication and recently approved DAAs.
Methods: Concomitant outpatient medication including over-the-counter medicine and herbals was assessed in 261 unselected HCV-monoinfected pts that were evaluated for antiviral treatment at Hannover Medical School between 2011–2014.
DDI between the regular outpatient medication and the DAAs SOF, SMV, DCV and LDV/SOF were evaluated using the website www.hep-druginteractions.org and the prescribing information of all analyzed drugs. DDIs were classified in four different categories (0=Classification not possible due to lack of information, 1=no significant interactions, 2=moderate interaction, may need dose adjustment/monitoring, 3=co-administration not recommended).
Results: The 261 pts took 188 different drugs with a median of 2 drugs per patient (range 0–15). Importantly, only one drug (eslicarbazepine) (0.5%) taken by one patient (0.4%) was a category 3 drug for all four DAAs. Monitoring/dose adjustment (category 2) was recommended in the case of LDV/SOF for 18 drugs (10%) affecting 127 pts (49%). For SOF this was 5 drugs (3%) affecting 25 pts (10%), for SMV 30 drugs (16%) in 101 pts (39%) and DCV 20 drugs (11%) in 116 pts (44%).
Drugs taken for cardiovascular diseases (ATC C) were frequently affected by DDI with 16 of 41 drugs (39%) showing a potentially significant interaction with at least 1 of the four DAAs. Drugs taken for diseases of the nervous system (ATC N) showed DDIs in 11 of 36 drugs (31%).
DDI could not be assessed due to lack of information for 32 drugs (17%) in 72 pts (28%). Eighteen of these drugs (56%) were herbals or nutritional supplementations.
Conclusions: Treatment with recently approved DAA is associated with a low potential for serious DDI (category 3). However, moderate DDI are frequent and have to be considered in order to prevent adverse effects or a lack of antiviral efficacy.

P0755
HEPATITIS C INFECTION IS ASSOCIATED WITH AN INCREASE IN CARDIOVASCULAR DISEASES

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Background and Aims: Chronic Hepatitis C virus (HCV) infection is associated with insulin resistance, diabetes mellitus and systemic inflammation, which predisposes to atherosclerosis that may increase cardiovascular events.
The aim of this study is to explore the association of HCV infection and cardiovascular events in and analyze the impact of HCV on cost and length of stay in patients with cardiovascular disorders.
Methods: We used the weighed 2011 Nationwide Inpatient Sample (NIS) data to characterize patients with HCV infections. We identified all patients who had a cardiovascular event [Acute myocardial infarction (AMI) n = 201,950, coronary artery disease (CAD) n = 1,454,012, congestive heart failure (CHF) n = 944,870 and cerebrovascular accident (CVA) n = 53,623] in their primary discharge diagnosis for the 2011 data. Patients were compared based on their exposure (HCV) status in relation to cardiovascular outcomes.
Results: The inpatient prevalence of diagnosed HCV infection was 1.9%. Patients with HCV infection tended to be younger, poorer, male, smokers, alcohol consumers diabetic, minorities and more likely to die in the hospital compared to those without diagnosed HCV.
For patients with HCV infection, the adjusted odds ratio was for AMI (OR: 2.29, CI: 2.22–2.36), CAD: (OR: 1.88, CI: 1.83–1.93), CVA (OR: 1.98, CI: 1.93–2.04) and congestive heart failure: OR 1.08 (1.06–1.10).
The inpatient treatment cost and length of hospital stay of HCV patients with cardiovascular morbidity were consistently and significantly higher than non-HCV patients.
Conclusions: HCV is associated with a higher risk of developing a cardiovascular outcome, increased cost of care and length of hospital stay. HCV need to be considered a cardiovascular risk factor.

P0756
HEPATITIS C REINFECTION RATES FOLLOWING TREATMENT INDUCED SVR IN PATIENTS WHO ACTIVELY INJECT DRUGS AT THE TIME OF TREATMENT

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Background and Aims: Data on reinfection rates following treatment induced SVR in actively injecting drug users warrants further clarification1. This was the main aim of our study. Actively injecting drug users are often excluded from treatment or participation in clinical trials. One main barrier is the perception that they will be reinfected with hepatitis C2.
Methods: This is a prospective study looking at reinfection rates in a cohort of intravenous drug users that have been treated and achieved SVR with pegylated interferon and ribavirin. Potential participants were identified from our hepatitis C database. A group was identified who were actively injecting in the six months prior to treatment. This group included those who continued to inject during treatment. These are the 'current' drug using group. A second group that stopped injecting at least six months prior to treatment were identified. These are the 'past' drug using group. Information was collected on different outcomes including reinfection rates using annual HCV RNA (with subsequent genotyping if positive) and liver disease progression using liver function blood tests and fibroscan.
Results: Sixty patients were recruited, 36 classified as 'current' IDUs at the time of treatment and 24 'past' IDUs. There were two cases of reinfection in the 'current' group and no cases in the 'past' group. Both cases of reinfection were with a new genotype. The time between SVR and reinfection was 61 months in case one and 30 months in case two. The total duration of patient follow up was 245.9 years. The rate of reinfection was 0.81 cases per 100 patient years. If we examine only the 'current' IDU group, the rate of reinfection was 1.57 cases per 100 patient years.
Conclusions: This low rate of reinfection in current IDUs provides confidence as we move into the era of treatment with DAAs. Inclusion of current drug users in DAA programs is important to provide real life data on reinfection following SVR with these newer agents, in this group with the highest potential for onward transmission.

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P0757
EXPRESSION OF INHIBITORY CD158B AND CD158E NK CELL RECEPTORS IS ASSOCIATED WITH SIGNIFICANT LIVER INJURY IN THE COURSE OF CHRONIC HEPATITIS C IN CHILDREN

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Background and Aims: Although chronic hepatitis C (CHC) has relatively mild clinical course in children, some patients may develop significant liver lesions including liver cirrhosis still in childhood. Determination of such patients is extremely important for proper clinical management. NK cells play an important role in innate immune response and their high proportion is situated in the liver.
The aim of this study was to analyse possible relation between NK cells and the expression of their receptors and varied intensity of liver lesions in the course of CHC in children.
Methods: Study included 106 children with CHC – 54 boys and 52 girls, age 13.62±3.48 years. Blood for biochemical, virological and cytometric testing was taken prior to the liver biopsy. Histological evaluation was preformed according to METAVIR scoring system. NK cells was evaluated by flow cytometry.
Results: In 58 children with CHC (55.2%) significant liver fibrosis was observed assessed at least F2 in METAVIR scoring system. In 18 children (17.2%) advanced liver fibrosis was found. Number of NK cells was comparable in the subgroups of children with varied ALT activity, presence of significant and advanced fibrosis and liver steatosis. Higher proportion of cells expressing CD158e inhibitory receptors was observed in the group of children with ALT >2UNL (21.11±14.60 vs 12.22±8.99%; $p = 0.037$). While higher proportion of cells expressing inhibitory CD158b receptor was observed in children with advanced fibrosis ($F \geq 2$) compared to minimal fibrosis ($F < 2$) – 34.14±12.44 vs. 27.48±8.71%; $p = 0.049$. Similar findings regarded MFI of CD158b in children with advanced fibrosis ($F \geq 3$) compared to fibrosis <3 – 5344.20±3407.49 vs 2979.67±1190.64; $p = 0.049$. Number of NK cells with the expression of CD158b was found a predictor of significant fibrosis in univariate analysis – OR 1.065; 95% CI 1.07–1.15; $p = 0.046$.
Conclusions: Higher proportion of NK cells with the expression of inhibitory CD158b and CD158e receptors is associated with liver injury expressed as higher aminotransferase activity, more advanced liver fibrosis and the presence of steatosis.

P0758
THE RELATIONSHIP BETWEEN HEPATITIS C RELATED LIVER DISEASE WITH DIABETES MELLITUS AND VITAMIN D

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Background and Aims: The high prevalence of Diabetes mellitus and vitamin D deficiency among HCV patients is a cause for concern. The effect of diabetes mellitus and levels of 25-Hydroxy Vitamin D on cirrhosis, decompensation and HCC remains unclear in HCV patients. This study iis aimed to assess the influence of diabetes mellitus and levels of 25-Hydroxy Vitamin D on hepatitis C related liver disease patients
Methods: In this retrospective study, all patients who tested positive for Anti-HCV from December 2010 to August 2014 and presented to department of Gastroenterolgy and Hepatology at our center were included and were reviewed for gender, age, diabetes, genotype, level of 25-Hydroxy Vitamin D, status of liver disease including HCC at inclusion. The association of diabetes mellitus and low 25-Hydroxy Vitamin D levels (<30 ng/mL) with liver status and child pugh class (CTP) was analyzed
Results: A total of 777 patients were included in the study. At presentation 439 (56%) patients had cirrhosis; including 51 (7%) with HCC and 287 (37%) had chronic hepatitis. 25-Hydroxy Vitamin D level was available in 206 patients at baseline. Low 25-Hydroxy Vitamin D level was present in [126/206 (61%)] of the patients. Diabetes mellitus was present in [165/777 (21%)] of the patients. Diabetes was associated more commonly with genotype 1 and 4 [25% (35/139)] in comparison to genotype 2 and 3 [18% (59/325)] ($p = 0.056$). Patients who had associated diabetes presented more commonly with cirrhosis 122/439 (28%) and HCC 122/439 (29%) in comparison to chronic hepatitis 28/287 (10%) ($p < 0.01$). Lower 25-Hydroxy Vitamin D level (<30 ng/mL) was seen in 67% (98/147) of patients with cirrhosis, 78% (11/14) with HCC and 47% (28/59) with chronic hepatitis ($p = 0.012$). In cirrhotic patients vitamin D deficiency was significant in child B 72% (52/72) and Child C 75% (12/16) patients in comparison to child A patients 52% (62/118) ($p = 0.013$). No significant relation of diabetes with child class was seen.
Conclusions: 25-Hydroxy Vitamin D deficiency was present in 61% and diabetes mellitus co-existed in 21% of HCV patients at presentation. Diabetes and low levels of 25-OH vitamin D at presentation was associated with higher incidence of cirrhosis and HCC. Lower 25-Hydroxy Vitamin D levels also leads to a higher incidence of decompensation. Improving diabetes control and correcting vitamin D deficiency can improve outcomes in hepatitis C related liver disease.

P0759
HEPATITIS C VIRUS INFECTION SELECTIVELY AFFECTS "EXECUTIVE FUNCTIONS" IN PATIENTS WITH CHRONIC NON ADVANCED LIVER DISEASE

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Background and Aims: Growing evidence suggests that patients with chronic Hepatitis C virus (HCV) infection develop neurocognitive deficits unrelated to staging of liver disease, indices of liver dysfunction, viral load, or genotype. Early symptoms of mental impairment have been described as "brain fog" or problems with attending to and recalling everyday information. However, until now, no study performed a comprehensive evaluation of the HCV infected patient's cognitive status by anlysing all specific neurologic domains, *i.e.*, memory, attention, problem solving, language, visuospatial, processing speed motor, and emotion.
We aimed to analyze the neuropsychological and cognitive functions in patients with chronic HCV genotype 1 infection without cirrhosis by using standardized neuropsychological tests validated in clinical practice.
Methods: Thirty patients with histologically proved HCV genotype 1b chronic hepatitis, naive to antiviral therapy were enrolled. Exclusion criteria were: causes of concomitant liver disease (such as HBV infection, autoimmunity or alcohol abuse), clinical, laboratory or histological signs of liver cirrhosis, <4 years of education, major depression or other psychiatric disorders, vitamin B12 and/or folate deficiency, hypothyroidism.

All patients were evaluated biochemically and underwent standardized neuropsychological tests: Wechsler Adult Intelligence Scale (WAIS-R) for the assessment of intelligence quotient (IQ), Corsi test to explore the visual-spatial memory, *attentional matrices for the assessment of attentional capacity* and trail-making test A and B (TMT-A and TMT-B) for the evaluation of psychomotor speed, attention and concentration.
Results: WAIS-R, which synthesizes the intellectual functioning, was in the average range expected in the general population (100±20). Corsi test and attentional matrices also showed no different scores from the expected average. TMT-A and TMT-B significantly deviated from the average values in 25% and 38% of the patient, respectively. While in the first case the results were independent of educational level, clinical, laboratory, virological and histological variables, in the latter there was a significant linear correlation with age.
Conclusions: Chronic HCV infection was associated with altered visual scanning, psychomotor speed, mental flexibility, and executive functions even if overall neurocognitive functions were preserved

P0760
PNPLA3 rs738409 I748M IS ASSOCIATED WITH STEATOHEPATITIS IN NON OBESE SUBJECTS WITH HEPATITIS C

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Background and Aims: The PNPLA3/Adiponutrin rs738409 C/G single nucleotide polymorphism is associated with the severity of steatosis, steatohepatitis and fibrosis in patients with non-alcoholic fatty liver disease, as well as with the severity of steatosis and fibrosis in patients with chronic hepatitis C (CHC). We tested in genotype 1 (G1)-CHC patients the putative association between the *PNPLA3* variant and histological features of steatohepatitis, as well as their impact on the severity of fibrosis.
Methods: 434 consecutively biopsied Caucasian G1-CHC patients were genotyped for *PNPLA3* rs738409. Histological features of steatohepatitis in CHC were assessed using the Bedossa classification. Hepatic expression of PNPLA3 mRNA was evaluated in 63 patients.
Results: The prevalence of steatohepatitis increased from 16.5% in patients with PNPLA3 CC, to 23.2% in CG and 29.2% in GG genotype ($p = 0.02$). By multiple logistic regression, PNPLA3 genotype (OR 1.54, 95% CI 1.03–2.30, $p = 0.03$) together with age (OR 1.03, 95% CI 1.00–1.05, $p = 0.02$), BMI ≥30 kg/m^2 (OR 2.06, 95% CI 1.04–4.10, $p = 0.03$) and HOMA (OR 1.18, 95% CI 1.04–1.32, $p = 0.006$) were independently linked to steatohepatitis. When stratifying for obesity, PNPLA3 was associated with NASH in non obese patients only (12.0% in CC vs 18.3% in CG vs 27.3% in GG, $p = 0.01$), including after correction for metabolic confounders (OR 2.06, 95% CI 1.26–3.36, $p = 0.004$). We confirmed the independent association of rs738409 with the severity of steatosis (OR 1.71, 95% CI 1.20–2.45, $p = 0.003$), and, indirectly by steatohepatitis promotion (OR 2.05, 95% CI 1.05–4.02, $p = 0.003$), with severe fibrosis. Higher liver PNPLA3 mRNA was associated both with the severity of steatosis (adjusted $p = 0.03$) and steatohepatitis after adjusting for gender, age, BMI and HOMA ($p = 0.002$).
Conclusions: In G1-CHC patients, the *PNPLA3* G variant is associated with a higher risk of steatosis severity and steatohepatitis in chronic hepatitis C, particularly among non obese subjects.

P0761
EPIDEMIOLOGICAL TRENDS AMONG PATIENTS WITH CHRONIC HCV INFECTION IN A TERTIARY CENTRE IN THE NETHERLANDS

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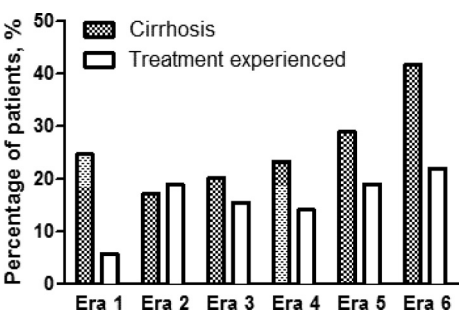
Background and Aims: The field of antiviral therapy for chronic HCV infection is rapidly evolving, with the introduction of very promising regimens. As these treatment regimens are genotype-specific, this study aimed to assess the epidemiological changes in patient and disease characteristics among patients with chronic HCV infection in a Dutch population.
Methods: All consecutive patients with chronic HCV mono-infection who were referred to the Erasmus MC University Medical Centre Rotterdam (EMC), a large tertiary centre in the Netherlands between 1990 and 2013 were included. To assess the trends in patient characteristics, the study population was divided in six eras based on date of first visit to the outpatient clinic (1990–1993, 1994–1997, 1998–2001, 2002–2005, 2006–2009 and 2010–2013).
Results: A total of 1779 patients were diagnosed with chronic HCV infection by positive HCV RNA. At time of referral mean age was 47 years (SD 12), 1210 (68%) were male and 459 (26%) had cirrhosis and 915 (51%) had genotype 1. From the total cohort, 1687 (79.1%) were born between 1940–1970. Age at referral increased over time with a mean age of 43.6 (SD 13.8) in era 1 and a mean age of 51.7 (SD11.2) in era 6 ($p < 0.001$). The number of patients who were referred with cirrhosis increased over the era's ($p < 0.001$), with respectively 31 (25%) patients in era 1 and 118 (42%) patients in era 6. In the first era, 7 (6%) treatment experienced patients were referred compared to 63 (22%) patients in era 6 ($p < 0.001$). Among patients with HCV genotype 1, 27 (48%) patients with subtype 1a and 29 (52%) patients with subtype 1b were referred in era 1 compared to 58 (54%) and 49 (46%) patients with respectively 1a and 1b in era 6 ($p = 0.001$). In the population of 454 patients who are currently visiting the outpatient clinic, 309 (68%) patients are male, 298 (66%) patients have genotype 1 [with 114 (38%) patients having subtype 1a and 94 (32%) patients subtype 1b] and 140 (31%) have cirrhosis.
Conclusions: Over the last 25 years, the HCV-infected population being referred to a tertiary center is aging, more often treatment experienced and more often affected by severe liver disease. Patients with HCV genotype 1 infection have formed a stable majority, with an increasing referral of patients with HCV subtype 1a. These results implicate increasing need for treatment in patients with genotype 1a, higher age and more severe liver disease and optimisation of treatment among these patients is warranted.



P0762

NON-RNA TESTING AMONG HEPATITIS C VIRUS ANTIBODY-POSITIVE PATIENTS IN A NATIONAL PUBLIC HEALTH INSURANCE CARE SYSTEM: PREVALENCE, CHARACTERISTICS AND PREDICTIVE FACTORS

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Background and Aims: Current treatment for hepatitis C virus (HCV) infection achieves high rates of sustained virological response. However, only a minority of infected patients receive treatment. A low adherence to diagnostic clinical guidelines which recommends HCV RNA testing after a positive HCV antibody test to define HCV active or past infection, impacts in appropriate evaluation. Our aim was to evaluate the prevalence, characteristics and predictive factors for non-RNA testing.
Methods: Laboratory records were retrospectively reviewed to identify consecutive tests for HCV antibodies between 1 January 2011 and 31 December 2012 for an area of 380,000 inhabitants. Positive tests were identified and patients' medical records reviewed until 1 August 2014 to determine HCV RNA testing and to assess demographic and clinical factors associated with non-RNA testing (patient demographics, comorbidities, physician specialty, health behaviours, HVB and HIV co-infection, low social support, and blood tests). Multivariate analysis was performed to predict non-RNA testing.
Results: Of the 37,700 tests for HCV-antibodies (29,169 subjects), 995 patients (3.4%) tested positive and of these, 347 (34.8%) patients (77.5% male, 47 years, range 11–88) did not receive RNA testing. Excluding 26 deaths that occurred during follow-up, in 71.3% of patients RNA was not determined by physician decision (48% primary care, 24% drug-abuse detoxification unit, 9% penitentiary center, 19% academic center). In the multiple logistic regression analysis presence of normal alanine aminotransferase (OR 2.40, 95% CI 1.10–5.26, p = 0.028) and comorbidities (OR 2.03, 95% CI 1.03–4.03, p = 0.041) were independent predictors for non-RNA testing. Only half of the patients (n = 157) had limitations for peg-interferon based treatment (7% severe comorbidity; 56% psychiatric disease, 6% epilepsy, 6% thrombocytopenia, 1% decompensated cirrhosis, 16% lack of social support and 8% over 65 years).
Conclusions: In our cohort one third of HCV antibody-positive patients did not receive RNA testing and appropriate evaluation of HCV infection status. Presence of normal alanine aminotransferase and comorbidities are associated with non-RNA testing. However, half of the patients were eligible to peg-interferon-based therapies. Multidisciplinary educational interventions are needed to reduce current barriers concerning appropriate evaluation of HCV to optimize treatment of chronic HCV infection.

P0763

HIGHER APRI PREDICTS EARLY SIGNIFICANT LIVER-RELATED EVENTS IN HEPATITIS C GENOTYPE 1 CHRONIC LIVER DISEASE

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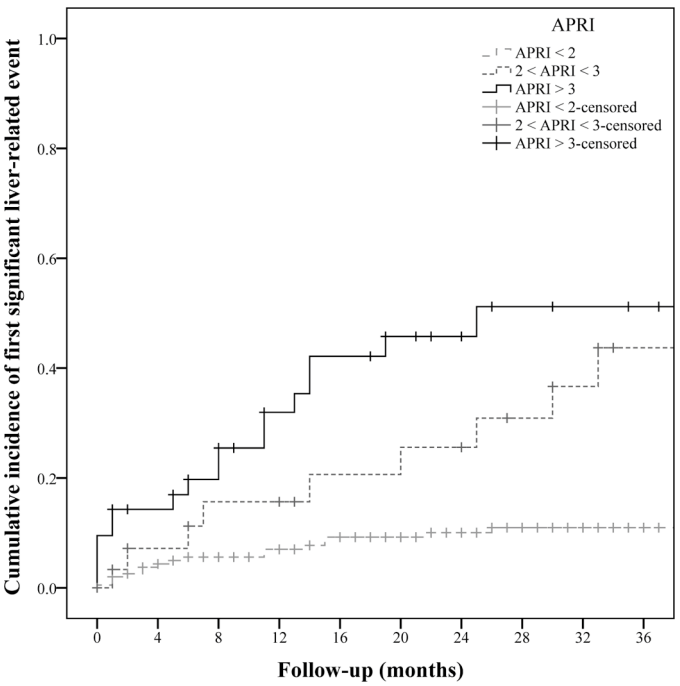
Background and Aims: Aspartate aminotransferase platelet ratio index (APRI) is highly sensitive and specific for predicting the presence of hepatic fibrosis, cirrhosis and portal hypertension in chronic hepatitis C (CHC). Nevertheless, the use of APRI in clinical practice remains under debate. Evaluate the prognostic accuracy of APRI in the prediction of liver-related events in a large cohort of CHC patients.
Methods: Retrospective observational study based on medical records from patients with a new diagnose of CHC genotype 1, followed at a tertiary center between January 2006 and December 2013.
Results: A total of 274 patients (65% males) were analyzed. Patients were followed for a mean time of 28 months (±24). At diagnosis, 36% of the patients had cirrhosis, of which 70% were Child–Pugh A. Twenty-five percent of the latter had decompensation. The median APRI was 0.987 [P_{25-75}: 0.568–2.124] and there was a positive correlation between APRI and METAVIR score ($p < 0.001$). The APRI was higher in patients with hepatocellular carcinoma (HCC) (2.060 [P_{25-75}: 1.017–3.636] *vs* 0.886 [P_{25-75}: 0.510–1.656], $p < 0.001$), ascites (2.299 [P_{25-75}: 1.584–4.487] *vs* 0.867 [P_{25-75}: 0.508–1.570], $p < 0.001$), encephalopathy (2.463 [P_{25-75}: 1.329–4.088] *vs* 0.907 [P_{25-75}: 0.511–1.756], $p = 0.009$), varices (2.675 [P_{25-75}: 1.633–4.269] *vs* 0.860 [P_{25-75}: 0.499–1.449], $p < 0.001$) and gastrointestinal bleeding (3.174 [P_{25-75}: 1.849–6.615] *vs* 0.906 [P_{25-75}: 0.512–1.750], $p < 0.001$). APRI higher than 1.250 predicted HCC with 67% sensitivity and 64% specificity; higher than 1.336 predicted cirrhosis with 73% sensitivity and 80% specificity; higher than 1.722 predicted varices with 73% sensitivity and 80% specificity; higher than 1.839 predicted ascites with 70% sensitivity and 80% specificity and encephalopathy with 70% sensitivity and 76% specificity; higher than 1.994 predicted gastrointestinal bleeding with 73% sensitivity and 79% specificity. The higher the APRI, the earlier the onset of complications in the disease follow-up, with the first significant liver-related event presenting later in patients with APRI lower than 2 than in patients with APRI between 2 and 3 and patients with APRI higher than 3 ($p_{Log\ Rank}$ <0.001; $p_{Breslow}$ <0.001).
Conclusions: APRI could be used as a rapid screening tool to allocate cirrhotic patients to specific risk categories, as well as a marker for a tighter surveillance interval in patients with higher APRI.



P0764

HEPATITIS C VIRUS INFECTION INDUCES AN "EARLY" SUBCLINICAL LEFT VENTRICULAR DISFUNCTION

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Background and Aims: Hepatitis C virus (HCV) infection has been associated with several extrahepatic manifestations in a variety of tissues and organs, including the myocardium. Reduced myocardial perfusion or increased pro-B-type natriuretic peptide (NT-proBNP) have been described in HCV-infected individuals but, until now, no study clearly demonstrated the occurrence of myocardial dysfunction in the early stages of HCV-related liver diseases.
Based on these premises, aim of this study was to evaluate the impact of HCV infection on left ventricular (LV) geometry and function by a standard 2D and 3D echocardiography.
Methods: Thirty consecutive patients with histologically proved HCV-related chronic hepatitis and twenty normal controls (NCs), comparable for age and gender prevalence were enrolled in the study. Exclusion criteria were: other causes of liver diseases, coronary artery and/or valvular heart disease, heart failure, cardiomyopathies, atrial fibrillation or alcohol abuse. The echocardiography protocol consisted of: standard echo Doppler plus LV volumetric and systolic function analysis through 3D echocardiography. Left ventricular systolic function thorough 3D echo was evaluated by STE derived global longitudinal strain (GLS), global circumferential strain (GCS), global area strain (GAS) and global radial strain (GRS).
Results: Overall we enrolled 16 males and 14 females with HCV (genotype 1) related chronic liver disease. Mean age was 60.9±6.5 yrs. NCs were comparable for age, gender distribution, body mass index, heart rate and blood pressure. In HCV infected subjects standard 2D echocardiography point out a transmitral E/A ratio marginally lower (0.84±0.2) compared to NC (1.01±0.3) (p = 0.04). Moreover, more detail 3D STE showed a subclinical alteration of LV function documented by lower GCS (HCV=-15.3±2.2%, NC=-17.6±3.9%, p = 0.02), GAS (−25.2±2.6%, NC −29.9±5.2%, p = 0.03), and GRS (37.6±4.6% vs 44.9±12%, p = 0.04) whereas HCV-releated changes of GLS were not significant.
Conclusions: HCV chronic infection is associated a subclinical myocarditis-like damage documented by the alteration of the circumferential fibers of the ventricular mid wall.

P0765

CONTINUUM OF CARE OF HEPATITIS C FROM DETECTION TO CURE: IMPACT OF PEER-TO-PEER SESSIONS IN PRIMARY CARE

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Background and Aims: To analyze the continuum of care in hepatitis C from primary care to academic hospital including the impact of peer-to-peer sessions in detection rate, referral rate and diagnostic and therapeutic management.
Methods: Peer-to-peer sessions were conducted in 13 of 26 Primary Care Centers (PCC) of our Area for Health Management. The sessions consisted in 1-hour of discussing epidemiological and clinical aspects of hepatitis C focusing on barriers to screening and referral of anti-HCV positive patients. The anti-HCV serology requested from PCC was analyzed (comparing trained centers versus non-trained centers) between 2008 and 2013 (n = 37846). The detection rate per 100000 attended population per year; the referral rate, the number of patients evaluated at the hospital and the number of patients treated were calculated. Statistical analysis was done by Chi2 test and t-student test using SPSS 20.0 (Chicago, IL).
Results: Detection rate of anti-HCV in trained centers was 52.7 per 100,000 population per year and 30.4 per 100,000 population per year in non-trained centers; p = 0.001 (OR 2.63; 95% CI: 1.47–4.7). The number of HCV positive patients was 3 times higher in trained centers (737 vs. 211) due to 3 times more requests. In multivariate analysis, age (OR 1.012; 95% CI: 1.01–1.015), male gender (OR 6.06; 95% CI: 5.20–7.04), number of peer-to-peer sessions (OR 1.58; 95% CI: 1.36–1.83) and trained (OR 2.307; 95% CI: 1.757–3.029) were independently associated with the possibility of detecting antiHCV positive cases. The highest prevalence of anti-HCV positive was in the group of men between 40–50 years (14.3%; 393/2350). The referral rate was 81.2% (770/948), with difference in absolute numbers in trained centers (598 vs. 172). Patients followed in the outpatient office in the Hospital were 733/948 (77%). Risk factors were: 197 drug users (27%), 176 transfused (24%), 42 tattoos (6%), 13 sexual (1.7%), 15 no disposable injections (2%), 11 vertical transmission (1.5%) and in 279 unknown (38%). Received antiviral therapy 203 patients (30%), 156 from trained centers (77%) and 47 from non-trained centers (23%).
Conclusions: Peer-to-peer sessions in primary care increased hepatitis C detection rate, which affected the number of referred and treated patients. The epidemiological profile of hepatitis C in our environment shows a concentration of cases in men between 30 and 60 years. These data support the need to implement peer-to-peer sessions in primary care to improve the continuum of care of hepatitis C.

Viral hepatitis: Hepatitis C – c. Clinical (therapy)

P0766

EPIDEMIOLOGY OF VIRAL RESISTANCE IN GENOTYPE 1 INFECTED PATIENTS AT APPROVAL OF IFN-FREE DAA COMBINATION THERAPY OF CHRONIC HEPATITIS C IN GERMANY

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Background and Aims: IFN-free direct antiviral agents (DAA)-based antiviral therapies including either a NS5A and/or a NS3 protease inhibitor are currently available for patients with chronic hepatitis C. In patients with pre-existence of resistance associated variants (RAVs) lower SVR rates have been observed especially in combination with other negative response predictors like liver cirrhosis, failure to PEG/R and failure to BOC/TVR-based previous antiviral therapy. A large number of patients with urgent need of antiviral

therapy have been treated with first generation protease inhibitors before. The rate of patients with RAVs to NS3 protease-, and NS5A-inhibitors at approval of DAA combination therapies is unknown.
Methods: Population based sequencing of NS3 and / or NS5A genes for identification of RAVs have been performed in a large number (n = 978) of treatment naïve and experienced HCV genotype 1 infected patients in Germany.
Results: Patients were either treatment-naïve (n = 97; GT1a n = 63, GT1b n = 34) or treatment experienced with PEG/R (n = 169; GT1a n = 104, GT1b n = 65), TVR+PEG/R (n = 463; GT1a n = 270, GT1b n = 193), or BOC+PEG/R (n = 249; GT1a n = 132, GT1b n = 117). For the NS3 protease the most frequent RAV observed in treatment naïve and PEG/R experienced patients was Q80K with 19.5–21% for GT1a and 1.1–1.2% for GT1b. In first generation protease inhibitor pre-treated patients RAVs at positions V36, R155, T54, A156 and V170 were most common. Here for TVR 19% V36 and 20% R155, 17% T54, 12% A156 and 12% V170 variants in GT1a and 8.8% V36, 9% A156 and 8% V170 variants in GT1b patients were observed while for BOC RAVs were less common with 2% V36, 12% R155, 7% A156 and 3% V170 in GT1a and 13% T54, 9% V170 and 10% V36 in GT1b. Frequencies of NS5A RAVs (L31, Y93, M28, Q30, L31, H58%, P58% and A92) did not differ between treatment-naïve and -experienced patients with 1.9% L31, 3.1% Y93, 3% M28, 2.7% Q30, 3.6% L31, 1.2% H58, 0.7% P58 and 0.6% A92.
Conclusions: A large number of patients with chronic hepatitis C and urgent need for antiviral therapy received first generation triple therapies. With the availability of all oral IFN-free therapies up to 36% and 7% of patients harbor RAVs within NS3 and NS5A genes which may affect treatment efficacy of protease- and NS5A-inhibitors.

P0767

CLINICAL UTILITY OF HEPATITIS C VIRUS CORE ANTIGEN TESTING IN THE MONITORING OF TREATMENT NAÏVE NON-CIRRHOTIC PATIENTS RECEIVING AN ALL-ORAL, INTERFERON-FREE REGIMIEN

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Background and Aims: Advances in the treatment of Hepatitis C virus (HCV) have demonstrated >90% Sustained Virologic Responses (SVR) after 12 weeks interferon-free, direct-acting combination therapy regardless of viral genotype, degree of liver fibrosis or previous treatment history. Simplification of treatment monitoring is required to ensure broad access to these new therapies. Introduction of these highly potent therapies have negated the role of response-guided therapy and reduced the role of treatment monitoring with highly sensitive HCV RNA tests. This study aims to demonstrate the utility of the HCV Core Ag (HCV Ag) test to accurately identify patients with active viremia and discriminate those who achieve SVR from those who fail therapy.
Methods: Samples from treatment naïve non-cirrhotic subjects with HCV genotype 1 infection enrolled in AbbVie Sapphire I clinical trial (ritonavir-boosted paritaprevir, ombitasvir and dasabuvir w/o ribavirin 12 weeks) were tested in a blinded fashion with Abbott HCV Ag test and results were later compared to the manual Roche High-Pure-System/COBAS® TaqMan (HPS) HCV RNA. The 451 samples tested in this study represented Baseline or Screening samples (N = 246), Day 1 samples (prior to first dose: N = 5), SVR 12 samples (N = 195), and 5 samples with last available time points (1 at SVR, 1 at SVR8, 1 each on treatment week 2, 8, and 10).
Results: Using 10 fMol/mL cutoff for HCV Ag detection, the HCV RNA and HCV Ag tests were in 100% agreement for truly negative samples and 99.6% agreement for truly positive samples. One discordant (screening) sample was identified. This sample was Target Not Detected by HCV RNA method but highly positive by HCV Ag (7912 fMol/mL). The mean (range) HCV Ag value was 7751 (25 to 66469) fMol/mL and 1.47 (0.5 to 8.4) fMol/mL for true positives and negative samples, respectively.
Conclusions: In this study HCV Ag, with a 10 fMol/mL cutoff, accurately identified 99.6% of patients with active viremia and discriminated all subjects who achieved SVR from those who failed therapy. In this context the HCV Core Antigen test (HCV Ag) is a useful tool in the identification and management of treatment naïve, non-cirrhotic patients with chronic HCV receiving all-oral, interferon-free therapy. 10 fMol/mL appears an appropriate clinical cutoff to determine active HCV viremia and discriminate SVR from non-SVR. HCV Core Antigen test can be used as an alternative to HCV RNA testing to improve global access to new HCV therapies.

P0768

LATE MORTALITY IN TREATMENT-EXPERIENCED CIRRHOTIC PATIENTS TREATED WITH TRIPLE THERAPY INCLUDING BOCEPREVIR OR TELAPREVIR IN A REAL-LIFE COHORT – ANRS CO 20 CUPIC

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Background and Aims: Sustained virological response (SVR) rate has increased with the use of triple therapy including

boceprevir (BOC) or telaprevir (TVR) in genotype (GT) 1 cirrhotic patients. However, the prognosis of these patients is not clearly established. The aim of this analysis was to determine the rate and the predictors of late mortality in patients included in the CUPIC cohort.

Methods: Between January 2011 and April 2013, 629 treatment-experienced GT 1 patients with compensated cirrhosis received triple therapy including BOC or TVR. We analysed rates and causes of death occurring before (early death) or after (late death) 12 weeks post-treatment of follow-up (FU). Baseline predictors of late death were determined.

Results: The median follow-up from the start of treatment was 30 months. Overall, 7% (44/629) of patients died, among them 29 (66%) died lately. The median interval time between FU12 and death was 15.7 months. Late deaths were related to hepatic events (10) and/or infections (4) in 48% (14/29). The SVR rate in the late death group was 34% (10/29) vs 48% in the survivor group (p = 0.5664 Log-rank). Baseline liver stiffness was available in 17/29 patients who died lately and in 334/585 survivors. The mean baseline liver stiffness was significantly higher in the dead group compared to the survivor group (31.5 vs 22 kPa, p = 0.03, Wilcoxon test). The baseline predictors of late death (uni and multivariate analysis adjusted on age and gender) are presented in the table. SVR12 status was not a predictor of death.

Conclusions: The 30 months mortality in GT 1 cirrhotic patients treated with triple therapy is high (7%). Two third of deaths occurred lately. In near of 50% of cases, late deaths were due to hepatic events or infection and were mostly associated with the severity of cirrhosis. SVR 12 was not an independent predictor of survival at 20 months but follow up is still limited.

	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
Hemoglobin	0.76	0.61–0.96	0.019	NS		
Platelets	0.99	0.98–0.10	0.025	NS		
Albumin	0.87	0.80–0.94	0.0006	0.89	0.81–0.98	0.021
Prothrombin time	0.97	0.95–0.99	0.0093	NT because too much missing data		
SVR12 yes/no	0.79	0.36–1.75	0.56	NS		
Liver stiffness	1.03	1.00–1.06	0.0181	NT because too much missing data		

P0769

EFFICACY OF AN EIGHT-WEEK REGIMEN OF GRAZOPREVIR PLUS ELBASVIR WITH AND WITHOUT RIBAVIRIN IN TREATMENT-NAIVE, NONCIRRHOTIC HCV GENOTYPE 1B INFECTION

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Background and Aims: The combination of grazoprevir (GZR; 100 mg) a highly potent NS3/4 inhibitor and elbasvir (EBR; 50 mg) an NS5A replication complex inhibitor exhibits a high barrier to resistance. In C-WORTHY parts A and B, GZR + EBR with or without ribavirin (RBV) demonstrated robust efficacy with >95% SVR_{12} in genotype (GT)1 HCV-infected patients (modified intent to treat) treated for 12 weeks. Since shorter treatment durations can improve adherence, reduce drug exposure and decrease cost, we assessed the safety and efficacy of an 8-week regimen of GZR + EBR ± RBV in treatment-naive, noncirrhotic patients with HCV GT1b infection.

Methods: 61 patients with HCV GT1b infection were randomized to GZR plus EBR with or without RBV for 8 weeks. Absence of cirrhosis was confirmed by either liver biopsy or noninvasive tests. The primary end point was the proportion of patients with HCV RNA <25 IU/mL 12 weeks after treatment (SVR12) assessed by COBAS TaqMan v2.0 (lower limit of quantitation <25 IU/mL).

	HCV GT1B infection	
	GZR + EBR + RBV (8 weeks) n = 30	GZR + EBR (8 weeks) n = 31
TW4 [a], n/N (%)	30/30 (100%)	30/31 (97%)
TW8 [a] (EOT), n/N (%)	30/30 (100%)	31/31 (100%)
SVR 4 [a], n/N (%)	29/30 (97%)	30/31 (97%)
SVR 12 [a], n/N (%)	27/29 [b] (93%)	29/31 (94%)
Breakthrough, n	0	0
Relapse, n	2	2

[a] Proportion of patients with HCV RNA <25 IU/mL (mITT, excluding 8 of 471 patients lost to follow-up or who discontinued early unrelated to adverse event or virologic failure).
[b] Excludes 1 patient who discontinued unrelated to adverse event or virologic failure.

Results: At baseline, the mean viral load was 6.4 $\log_{10}$ (4.4, 7.7), mean age was 53 years (range 28–71), 48% were male, 82% were white and 77% had *IL28B* non-CC. HCV RNA was undetectable after 4 weeks in 100% of patients treated with RBV and 97% without RBV (table). By end of treatment (EOT) at 8 weeks, 100% of patients had HCV RNA <25 IU/mL. Twelve weeks after EOT, 2 patients in each arm had relapsed. The most common adverse events (AEs) were fatigue, headache and nausea, occurring in 23%, 18% and 13%, respectively. No drug-related serious AEs or discontinuations due to AEs were reported.

Conclusions: Therapy for 8 weeks with GZR (100 mg) plus EBR (50 mg) ± RBV in treatment-naive, noncirrhotic HCV GT1b-infected patients was highly efficacious, safe and well tolerated.

P0770

EFFECT OF HEPATITIS C ANTIVIRAL THERAPY ON ALL-CAUSE MORTALITY AND DEVELOPMENT OF CANCER IN THE CHRONIC HEPATITIS COHORT STUDY (CHECS)

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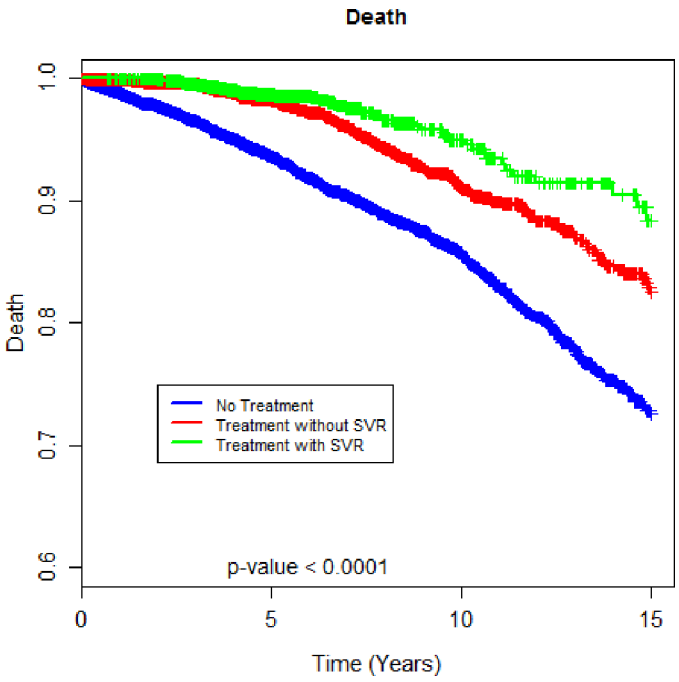
Background and Aims: We sought to study the effect of hepatitis C (HCV) antiviral therapy, with and without sustained viral response (SVR, HCV RNA negative 12 weeks after completion of therapy), on the incidence of (a) hepatocellular carcinoma (HCC), (b) non-liver cancer, and (c) all-cause mortality.

Methods: Data were drawn from the Chronic Hepatitis Cohort Study (CHeCS), an observational study from 4 large US health systems. Demographics, HCV genotype, FIB4, decompensated status, and comorbidities including diabetes and history of alcohol abuse were collected at the index date (defined as date of first HCV diagnosis or antiviral therapy). HCV treatment detail and virologic response were captured until end of follow-up or HCC, non-liver cancer, or death. Cox regression, univariate followed by multivariate, and propensity score were used to control for possible imbalances at index between treated and untreated groups. Sensitivity analyses were performed.

Results: A total of 9,143 patients were included with a median follow-up of 6.4 years; 2,855 (31%) were treated, of whom 39% achieved an SVR. Treatment reduced mortality (aHR = 0.40, 95% CI 0.28–0.56 for treated patients who achieved SVR; aHR = 0.69, 95% CI 0.56–0.85 for treated patients without SVR; see figure). Moderate-to-advanced fibrosis (FIB4 >1.21) was an independent risk factor both for HCC (aHR = 3.9, 95% CI 1.86–8.59) and death (aHR = 2.04, 95% CI 1.45–2.86). Patients with genotype 3 had a higher risk of HCC compared to genotype 1 (aHR = 1.8, 95% CI 1.15–2.84). Males had higher risk of both HCC (aHR = 2.94, 95% CI 2.12–4.16) and

death (aHR = 1.51, 95% CI 1.23–1.85). HCC screening was lower in the treated than the untreated group (0.20 vs. 0.28 screenings/person-year). Nevertheless, patients with SVR had a lower risk of HCC (aHR = 0.54, 95% CI 0.34–0.84) compared to treated patients without SVR, while treated patients without SVR had a higher risk of HCC than untreated patients (aHR = 1.97, 95% CI 1.48–2.63). Blacks had a higher risk of non-liver cancer compared to whites (aHR = 2.09, 95% CI 1.44–3.02), while treatment, with or without SVR, had no effect. Older age was a risk factor for all outcomes.

Conclusions: In a large, real world US multicenter cohort following both treated and untreated HCV patients, presence of moderate-to-advanced fibrosis and genotype 3 independently increased risk of HCC, while SVR reduced development of HCC by 46%. Treatment, regardless of whether SVR was attained, reduced all-cause mortality.



P0771

C-SCAPE: EFFICACY AND SAFETY OF 12 WEEKS OF GRAZOPREVIR +/− ELBASVIR +/− RIBAVIRIN IN PATIENTS WITH HCV GT2, 4, 5 OR 6 INFECTION

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Background and Aims: Grazoprevir (GZR; an NS3/4A protease inhibitor) and elbasvir (EBR; an NS5A inhibitor) are highly potent direct-acting antiviral agents (DAAs) with a high barrier to resistance, and shown to be highly effective for HCV genotype (GT)1 infection in the phase 2 C-WORTHY study. *In vitro*, both compounds have pan-genotypic properties. C-SCAPE is a phase 2 study investigating the efficacy and safety of once-daily GZR ± EBR given with ribavirin for 12 weeks in patients with HCV GT2 infection; and given as GZR + EBR ± ribavirin for 12 weeks in patients with HCV GT4, 5 or 6 infection.

Methods: HCV RNA was assessed using COBAS TaqMan v2.0 (LoQ 15 IU/mL). The primary end point was the proportion of patients with HCV RNA <25 IU/mL 12 weeks after end of treatment (SVR12).

Results: 100 patients were enrolled: 60% had GT2 infection, 20% GT4, 8% GT5 and 12% GT6. 57% were male, and 85% were white. Mean baseline viral load was 6.9 $\log_{10}$ IU/mL and all patients were noncirrhotic. Table 1 shows treatment regimens and efficacy. The first 30 patients with GT2 infection receiving GZR + EBR + RBV were assessed for a polymorphism of the virus based on preclinical data demonstrating a lower potency for EBR in methionine (M) compared with leucine (L) at amino position 31 within NS5A. Four of 30 patients had virologic failure; 4/13 patients with 31M and 0/14 patients with 31L. One patient with GT4 infection discontinued study drug at treatment week 8 with an asymptomatic ALT elevation >10×ULN possibly related to study drug confounded by the concurrent use of etifoxine (a known hepatotoxic drug). Other mild to moderate drug-related adverse events included fatigue (26%), headache (21%) and asthenia (16%).

Conclusions: These data demonstrate high efficacy for patients with HCV GT2 (31L), GT4 and GT6 infection and lower efficacy for patients with GT2 (31 non-L) or GT5 (treated without RBV) infection. These data support the inclusion of patients with GT4 or GT6 infection in the phase 3 GZR/EBR program.

Regimen	Genotype	N	EOT [a]	SVR12 [a]
GZR+EBR+RBV	2	30	93% (28/30)	80% (24/30)
GZR+RBV		30	80% (24/30)	67% (20/30)
GZR+EBR+RBV	4	10	100% (10/10)	100% (10/10)
GZR+EBR		10	90% (9/10)	90% (9/10)
GZR+EBR+RBV	5	4	100% (4/4)	100% (4/4)
GZR+EBR		4	75% (3/4)	25% (1/4)
GZR+EBR+RBV	6	5	100% (5/5)	80% (4/5)
GZR+EBR		5	80% (4/5)	80% (4/5)

EBR, elbasvir; GZR, grazoprevir; RBV, ribavirin.
[a] Proportion of patients with HCV RNA <25 IU/mL.

P0772

DACLATASVIR PLUS SOFOSBUVIR WITH OR WITHOUT RIBAVIRIN FOR THE TREATMENT OF HCV IN PATIENTS WITH SEVERE LIVER DISEASE: INTERIM RESULTS OF A MULTICENTER COMPASSIONATE USE PROGRAM

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Background and Aims: The all-oral antiviral regimen using daclatasvir (DCV) + sofosbuvir (SOF) with/without ribavirin (RBV) has demonstrated high response rates along with minimal adverse events (AE) in treatment-naive patients (pts) with hepatitis C genotype (GT) 1, 2 and 3 and prior nonresponders to protease inhibitor-based therapies (GT1). Data in a real world setting especially in difficult-to-treat populations are clinically relevant.

This analysis reports interim findings from a compassionate use program (CUP) of DCV+SOF +/− RBV opened in 5 European countries in pts with severe liver disease.

Methods: This multicenter CUP (ClinicalTrials.gov, NCT02097966) enrolls adult pts with chronic HCV infection who are at a high risk of hepatic decompensation or death within 12 months if left untreated, and who had no available treatment options. Patients received DCV 60 mg + SOF 400 mg QD for 24 weeks, with RBV added at the physician's discretion. This interim analysis includes 413 pts with available data at October 3rd, 2014.

Results: The majority of pts were male (n = 281, 68%), Caucasian (n = 390, 95%), and most had non-CC *IL28B* (140 of 170 pts with available data). Median age was 57 years (range, 27–83). Most pts were infected with HCV GT1 (GT1a, n = 131; GT1b, n = 157) and GT3 (n = 83), with 2 pts GT2, 15 pts GT4, and 1 pt GT5. A total of 42 pts had HIV/HCV coinfection, and 72 were liver transplant recipients. To date, liver biopsy (LB) and/or FibroScan (FS) results are available in 272 pts; of them, 196 had METAVIR F4 or FS >14.6 (Child–Pugh A, n = 111; B, n = 77; C, n = 8). On treatment virological response at Week 12 shows HCV RNA values <LLOQ TD/TND in 193 pts (93%) of 208 pts with available data. Six pts discontinued treatment due to AEs; 3 were considered treatment related (acute renal failure, n = 1; hepatic/renal failure, n = 1; hypersensitivity vasculitis, n = 1). Grade 3/4 laboratory abnormalities were infrequent. Nine pts died (all events were considered non-treatment related). Additional data (including sustained virological response results) will be available for presentation.

Conclusions: In this preliminary analysis, DCV+SOF +/− RBV demonstrated antiviral activity at Wk 12 and was well tolerated in difficult-to-treat pts with severe liver disease.

P0773

THE PREVALENCE AND THE EFFECT OF HCV NS5A RESISTANCE ASSOCIATED VARIANTS IN SUBJECTS WITH COMPENSATED CIRRHOSIS TREATED WITH LEDIPASVIR/SOFOSBUVIR +/− RBV

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Background and Aims: Here we evaluated the effect of baseline HCV NS5A resistance-associated variants (RAVs) on treatment outcome in 511 cirrhotic subjects treated with ledipasvir/sofosbuvir (LDV/SOF) ± ribavirin (RBV).

Methods: Population (n = 34) or deep (n = 477) sequencing for the HCV NS5A gene was performed at baseline (BL) for all enrolled subjects with cirrhosis in the phase 2 and 3 studies (LONESTAR, ELECTRON, ELECTRON-2, GS-US-337-0113, ION-1, 2 and SIRIUS) and for NS5B at BL in a subset of subjects by deep sequencing (n = 418). Consensus sequences were generated from deep sequences and resistance analysis was performed using 1% and 15% cut-offs (% of total reads).

Results: With a 1% cut-off, 94/511 (18%) cirrhotic subjects were identified as having BL NS5A RAVs. SVR results by regimen and patient group are shown in the table.

	SVR12, % (n/N)			
	LDV/SOF, 12 weeks	LDV/SOF+RBV, 12 weeks	LDV/SOF, 24 weeks	LDV/SOF+RBV, 24 weeks
All subjects				
With NS5A RAVs	88 (23/26)	94 (32/34)	85 (17/20)	100 (14/14)
No NS5A RAVs	95 (86/91)	97 (164/169)	100 (113/113)	100 (44/44)
Treatment-naïve subjects				
With NS5A RAVs	90 (9/10)	100 (8/8)	86 (6/7)	100 (10/10)
No NS5A RAVs	100 (36/36)	97 (36/37)	100 (26/26)	100 (26/26)
Treatment-experienced subjects				
With NS5A RAVs	88 (14/16)	92 (24/26)	85 (11/13)	100 (4/4)
No NS5A RAVs	91 (50/55)	96 (128/133)	100 (87/87)	100 (18/18)

Conclusions: LDV/SOF regimens for 12 and 24 weeks showed high efficacy in treatment naïve cirrhotic subjects with GT1 infection even in the presence of HCV NS5A RAVs at BL.

P0774

LEDIPASVIR/SOFOSBUVIR WITH RIBAVIRIN IS SAFE IN >600 DECOMPENSATED AND POST LIVER TRANSPLANTATION PATIENTS WITH HCV INFECTION: AN INTEGRATED SAFETY ANALYSIS OF THE SOLAR 1 AND SOLAR 2 TRIALS

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Background and Aims: Patients with HCV who have decompensated liver disease or who have recurrent HCV post liver transplantation have substantial rates of morbidity and mortality. The safety of any HCV treatment regimen is therefore critical to evaluate in order to determine if, when and who to treat. The safety of ledipasvir/sofosbuvir (LDV/SOF) + ribavirin (RBV) was evaluated in a pooled analysis of two studies enrolling patients with advanced liver disease in the US (SOLAR 1, NCT01938430) and Europe, Canada, Australia and New Zealand (SOLAR 2, NCT02010255), the largest study of such patients to be evaluated to date.

Methods: Six groups of patients with HCV genotypes 1 or 4 and decompensated liver disease or who were post liver transplantation were randomized to receive 12 or 24 weeks of LDV/SOF + RBV treatment: patients without transplant and either (1) Child–Pugh–Turcotte (CPT) B cirrhosis, or (2) CPT C cirrhosis; or patients who have undergone transplantation and who were either (3) without cirrhosis (F0 to F3), (4) CPT A cirrhosis, (5) CPT B cirrhosis, or (6) CPT C cirrhosis. RBV was administered either at 600 mg/day and escalated as tolerated (decompensated) or weight-based (1000–1200 mg/day; F0–F3, CPT A).

Results: 658 patients were randomized. 392 had HCV genotype 1a, 224 had HCV genotype 1b, and 42 had HCV genotype 4. The major safety findings are included in the table. Of 134 SAEs, only 20 were related to treatment, 11/20 (55%) were due to RBV-associated anemia. RBV-associated anemia was also the most common Grade 3 laboratory AE. There were 18 treatment-emergent (TE) deaths; sepsis (5), GI bleed (3), and myocardial infarct (3) occurred in more than 1 person and none were attributed to LDV/SOF treatment.

Conclusions: In these patient populations treatment with LDV/SOF + RBV was generally safe and well tolerated, irrespective of the degree of decompensation or whether patients were pre- or post-transplantation. The adverse events observed were consistent

with the disease status of the patients; no additional morbidity or mortality, aside from RBV-associated side effects, was associated with treatment. A detailed analysis of RBV dose modification and related safety events for this all oral regimen will be presented.

	Advanced liver disease pre-transplantation (N = 215)		Post-transplantation (N = 443)				Total, n (%) (N = 658)
	CPT B	CPT C	F0–F3	CPT A	CPT B	CPT C	
N	117	98	211	118	96	18	658 (100)
Any AE	113	94	203	107	91	17	625 (95)
≥Grade 3 AE	17	33	43	25	22	6	146 (22)
Related AE	89	68	174	89	72	11	503 (76)
SAE	22	35	29	15	26	7	134 (20)
Related SAE	3	4	5	6	2	0	20 (3)
AE leading to D/C	3	4	2	2	3	1	15 (2)
OLT	4	7	0	0	0	0	11 (2)
Death	3	7	0	3	3	2	18 (3)
HB <10 g/dL	20	34	93	51	46	11	255 (39)
HB <8.5 g/dL	5	10	31	19	15	2	82 (12)

P0775

FINAL EVALUATION OF 955 HCV PATIENTS TREATED WITH 12 WEEK REGIMENS CONTAINING SOFOSBUVIR +/− SIMEPREVIR IN THE TRIO NETWORK: ACADEMIC AND COMMUNITY TREATMENT OF A REAL-WORLD, HETEROGENEOUS POPULATION

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Background and Aims: Outcomes in a heterogeneous real-world population of hepatitis C (HCV) patients typically fail to match those observed through highly controlled clinical trials. To bridge the knowledge gap between investigation and application, ongoing studies of actual treatment are important to determine clinical effectiveness for patients infected with HCV. Trio Health is a disease management company that works in partnership with academic medical centers, community physicians and specialty pharmacies in the US to optimize care for HCV. Data obtained through the Trio Health program were analysed to evaluate efficacy of 12 week sofosbuvir +/− simeprevir regimens in a real-world HCV population.
Methods: Data were collected from Rx records through the Trio Platform in partnership with specialty pharmacies and was limited to 955 patients who initiated a 12 week course of treatment between Dec 2013 and Mar 2014. Outcomes from 822 patients were previously reported though 133 patients that were not included in these figures will be presented as part of the final evaluation of 955 patients. Treatment commenced in 142 clinics, 30 of which were academic.
Results: Mean age was 57 with 183 patients (19%) 65 years or older, 59% male and mean BMI 28.7. Genotype 1 was seen in 703 patients (74%), genotype 2 in 212 patients (22%), genotype 3 in 7 patients (1%), genotypes 4–6 in 25 patients (3%), and an unknown genotype for 8 patients (1%). Of the 955 patients, 16% were black. HIV co-infection was present in 5% of the population. 16% of patients had a baseline platelet measure of <100k/ul. Baseline Viral load >6MM IU/ml was seen in 19% of patients and ≤800K IU/ml was present in 32% of patients. 43% of patients were previously treated; 35% of which were null responders. Cirrhosis was present in 30% of patients. Treatment evaluation of 955 patients indicates an overall discontinuation rate of 5%. SVR12 rates from 822 patients were previously reported as 79% (Intent to Treat) and 88% (Per Protocol).
Conclusions: Final results, so far paralleling those of clinical trials, of outcomes for a heterogeneous population of 955 patients and sub-groups treated with 12-week regimens containing sofosbuvir +/− simeprevir will be presented.

P0776

EFFICACY OF 12 OR 18 WEEKS OF GRAZOPREVIR PLUS ELBASVIR WITH RIBAVIRIN IN TREATMENT-NAIVE, NONCIRRHOTIC HCV GENOTYPE 3-INFECTED PATIENTS

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Background and Aims: Grazoprevir (GZR) and elbasvir (EBR) are potent NS3/4A protease and NS5A replication complex inhibitors, respectively with pan-genotypic activity in vitro. In patients with HCV genotype (GT)1 infection enrolled in C-WORTHY (parts A B), GZR (100 mg QD) and EBR (50 mg QD) +/− ribavirin (RBV) demonstrated robust efficacy with >95% SVR12 (modified intent to treat). In a phase 1b study, patients with HCV GT3 infection treated with monotherapy EBR (50 mg for 5 days) or GZR (100 mg for 7 days) achieved a >3 log HCV RNA decline. These results supported the initiation of this phase 2 pilot study (PN035 Part D) to assess the safety and efficacy of the combination regimen in treatment-naive, noncirrhotic patients with GT3 infection.
Methods: Treatment-naive, noncirrhotic patients with GT3 infection were randomized to 12 (n = 21) or 18 weeks (n = 20) of GZR (100 mg) + EBR (50 mg) + RBV. The primary end point was HCV RNA <25 IU/mL 12 weeks after end of treatment (SVR12) assessed by COBAS TaqMan v2.0 (lower limit of quantitation <25 IU/mL). Cirrhosis status was determined by either liver biopsy or noninvasive tests.
Results: The study is ongoing: data as of 11/21/2014. Forty-one patients were enrolled: 39% were male, 93% were white, mean age was 46.0 years (range 22–61), and mean viral load was 6.0 $\log_{10}$ (range 2.7–7.4). In the 12-week arm, 10 patients experienced breakthrough or rebound during treatment, 1 patient relapsed and 10 patients achieved SVR4. In the 18-week arm, 7 patients experienced breakthrough or rebound during treatment, 1 patient discontinued unrelated to drug-related adverse event or virologic failure. Of the remaining 12 patients, 1 is TND (HCV RNA target not detected) at follow-up week 4 and 11 are TDu (HCV RNA target detected, unquantifiable, n = 1) or TND (n = 10) on treatment between weeks 12 and 18. Adverse events reported in >10% of patients were fatigue (25%), headache (24%) and asthenia (14%). Final SVR12 results will be presented.
Conclusions: In this pilot study of GZR (100 mg) + EBR (50 mg) + RBV in treatment-naive, noncirrhotic patients with HCV GT3 infection, low efficacy was achieved regardless of duration. Breakthrough or rebound occurred in 17 of 41 patients (41%). GZR and EBR with RBV is inadequate to treat GT3. Further study of this combination will be considered using a triple-drug regimen using GZR, MK-3682 plus either EBR or MK-8408, a pan-genotypic NS5A inhibitor with better GT3 activity.

P0777

SOFOSBUVIR-BASED TREATMENT UNDER REAL LIFE CONDITIONS IN GERMANY (THE SOFGER TRIAL)

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Background and Aims: The approval of direct-acting antivirals (DAA) in Germany starting with Sofosbuvir (SOF) in January 2014, Simeprevir (SIM) in May 2014 and Daclatasvir (DCV) in August changed the landscape of HCV-therapy. Recommendations in Germany (BNG/DGVS) were rapidly changed accordingly: first in favour for triple therapy with PEG-IFN, Ribavirin (Riba), SOF, later on for dual therapy with SOF/Riba (GT 2) and SIM/SOF (limited to special cases), and DCV/SOF. Aim of this study was to evaluate the change of treatment modalities following the implementation of recommendation as well as the safety and efficacy of the SOF based therapies under real life conditions.
Methods: In this non-interventional, prospective, multi-center study conducted by the Association of German Gastroenterologists in private practice (BNG) (5 centers) and one academic based center (Frankfurt), SOF-based therapies are currently being evaluated in Germany. Demographic, clinical, virology data and adverse events are collected throughout treatment and post-treatment. This interim analysis shows data about patients with SOF-based treatments from January to October 2014.
Results: 556 patients (319 male, 237 female) received SOF-based treatments. 339 pts with genotype 1 (152 1a, 187 1b), 61 genotype 2, 111 genotype 3, 45 genotype 4. Treatment-naive were 186, 352 had previous treatment (relapse 187, nonresponse 158, 18 unknown), 57% of patients had fibrosis F3–4. 58 pts received SOF/Riba for 12 weeks (all G2), 52 SOF+Riba for 24 weeks. 158 received triple therapy 12 weeks, 181 SOF+DCV +/− Riba (mainly F4, some in compassionate use), 5 SOF+SIM, 102 SIM/SOF. Within 5 months the landscape switched to mostly IFN-free regimen. SVR 12 data in the Triple-therapy group are available so far from 132 patients: 121 SVR12, 7 relapses, 4 pts were lost to follow up. 4 patients with IFN based therapy had to stop treatment because of IFN side effects. Anemia and fatigue were the most reported AEs. No severe side effects occurred. There were 4 deaths (out of 556) reported due to complications of cirrhosis. More SVR 12 data and detailed results for the different treatments will be available at the meeting.
Conclusions: SOF-based triple therapies under real life conditions seems to have comparable results to clinical trials. Following recommendations, there was a fast switch to IFN-free therapies in Germany in 2014. There is a clear trend to treat advanced patients first, but almost 40% were treated without advanced fibrosis. Adverse events and treatment discontinuations were low.

P0778

THE EFFECT OF HCV ANTIVIRAL THERAPY ON FIBROSIS PROGRESSION

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Background and Aims: The extent to which antiviral therapy for hepatitis C virus (HCV) infection alters the course of fibrosis progression is not well understood. We sought to study the impact of antiviral therapy, with and without sustained viral response (SVR), on fibrosis progression as measured by FIB4 trajectory among patients in the Chronic Hepatitis Cohort Study (CHeCS), a longitudinal observational study that draws patients from 4 large US health systems.
Methods: The index date was defined as date of HCV diagnosis or initial treatment, whichever was later. Patients were excluded if they were ever enrolled in an HCV treatment clinical trial, were co-infected with HBV, had received a liver transplant prior to the index date, or were receiving ongoing HCV treatment at end of follow-up. FIB4 scores were derived from routine testing and summarized as median values in 90-day intervals for up to six years after the index date. Only patients with two or more summarized FIB4 scores in addition to index date were included. Multilevel regression for longitudinal logFIB4, univariate followed by the multivariable modeling, was used to test the effects of HCV treatment and SVR on FIB4 trajectory over time. All analyses were adjusted using propensity score. Age, sex, FIB4, diabetes, and history of alcohol abuse at baseline were considered in the propensity score calculation.
Results: A total of 4,148 patients with confirmed HCV infection were included; 1,403 (34%) were treated (92% with dual ribavirin/interferon therapy and 5% with triple therapy), of whom 648 (46%) achieved an SVR. The final multilevel regression model showed significant change in FIB4 based on time, as well as interaction terms indicating that the fibrosis trajectory differed based on sex and timing of treatment response. Over time, men demonstrated consistently higher FIB4 levels than women ($p < 0.01$). For both males and females who achieved SVR, logFIB4 decreased for several years, then leveled off at year 4.5. Non-SVR responders demonstrated slow climbing logFIB4 in the range of 0.5–0.8 for females and 0.7–1.1 for males, while untreated males showed more rapid logFIB4 progression.
Conclusions: Eradication of HCV with antiviral therapy appeared to induce a regression of fibrosis, as measured by the FIB4 biomarker over a 6-year timeframe in a large diverse US cohort. Both absence of treatment and unsuccessful treatment were characterized by a progressive increase in fibrosis progression, with untreated males showing more rapid progression.

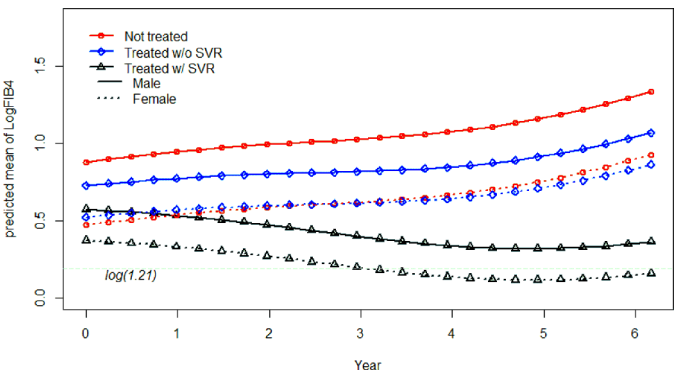

Figure: Predicted log FIB4 over time.

P0779

LEDIPASVIR/SOFOSBUVIR WITH RIBAVIRIN FOR THE TREATMENT OF FIBROSING CHOLESTATIC HEPATITIS C AFTER LIVER TRANSPLANTATION

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Background and Aims: Fibrosing cholestatic hepatitis (FCH) is a rare and severe form of recurrent hepatitis post liver transplantation with high morbidity (e.g., graft loss) and mortality. There are no approved treatments for FCH. This study evaluated ledipasvir/sofosbuvir (LDV/SOF) with ribavirin (RBV) in patients with biopsy-proven FCH.

Methods: In this open-label study, genotype 1 HCV-infected patients within 18 months of transplant, with histologic evidence of FCH (hepatocyte ballooning, periportal or pericellular/sinusoidal fibrosis, cholestasis, cholangiolar proliferation) total bilirubin ≥2.5×ULN and no alternative explanations for cholestasis, were randomized to receive 12 or 24 weeks of LDV/SOF with RBV (weight-based). The primary endpoint is SVR12.

Results: 12 patients have been randomized and treated. Most were male (75%), Caucasian (83%), GT 1a (75%) and had prior HCV treatment (75%). Mean baseline HCV RNA was 6.8 log10 IU/mL [range 4.9–8.0 log10 IU/mL]. 9 patients have completed treatment and 3 are still receiving treatment. There have been no treatment discontinuations. 5 patients (42%) experienced treatment-emergent serious adverse events (SAEs). One SAE, dyspnea, was considered related to study treatment. The most common adverse events were anemia, headache, fatigue and pruritus. Laboratory values at baseline and Week 12 are presented in the table. Bilirubin, GGT and alkaline phosphatase (hallmarks of FCH), as well as albumin, platelets, ALT and INR were all improving with treatment by Week 12.

By on-treatment week 4, eleven of 12 patients had HCV RNA <LLOQ and all 8 of the patients who have reached follow-up week 4 have achieved SVR4. SVR12 data will be presented.

	Normal range	LDV/SOF+RBV (N = 12)	
		Mean, baseline	Mean, Week 12
Bilirubin, mg/dL (range)	0.2–1.2	9.4 (1.5–28.1)	1.7 (0.6–5.2)
GGT, U/L (range)	4–61	554 (47–1332)	139 (15–686)
Alkaline phosphatase, U/L (range)	31–131	218 (114–562)	115 (40–356)
ALT, IU/mL (range)	6–43	161 (62–220)	30 (8–100)
Hemoglobin, g/dL (range)	11.5–18.1	12.5 (8.8–14.5)	11.6 (8.8–13.5)
Platelets $\times 10^3$/uL (range)	130–400	140 (40–217)	165 (53–280)
Albumin, g/dL (range)	3.3–4.9	3.0 (2.2–3.8)	3.7 (2.7–4.6)
INR (range)	0.8–1.2	1.4 (0.9–3.6)	1.1 (0.9–1.3)

Conclusions: Administration of LDV/SOF+RBV in patients with FCH has been well tolerated and resulted in high SVR4 rates in a population with no other options.

P0780

DEEP SEQUENCING ANALYSES IN HCV GENOTYPE 1-INFECTED PATIENTS TREATED WITH SIMEPREVIR PLUS SOFOSBUVIR WITH/WITHOUT RIBAVIRIN IN THE COSMOS STUDY

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Background and Aims: Simeprevir (SMV) and sofosbuvir (SOF) are approved for the treatment of chronic hepatitis C virus (HCV) infection. The presence of minority NS3/NS5B baseline polymorphisms and emerging mutations in HCV genotype 1-infected patients treated with SMV plus SOF with/without ribavirin in the COSMOS study (TMC435HPC2002; NCT01466790) was determined by deep sequencing (DS).

Methods: Illumina® NS3/NS5B DS data (1% cut-off) were available pre-treatment for 73/167 patients and post-baseline for 4/6 relapsers. In addition, early on-treatment NS3 DS data (Day 2–4 with HCV RNA >10,000 IU/mL) were available for 15 patients (including 2 relapsers). Changes at NS3 amino acid positions 43, 80, 122, 155, 156 and 168, and NS5B positions 96, 142, 159, 282, 316, 320, 321, 390 and 415 were analysed.

Results: Minority NS3 and NS5B baseline polymorphisms (i.e., not detected by population sequencing [PS]) were observed in 17.8% (13/73) and 11.0% (8/73) of patients, respectively. In 4/13 and none of the 8 patients carrying minority NS3 and NS5B polymorphisms, these variants were known to affect *in vitro* SMV or SOF activity, respectively. NS3 baseline polymorphism Q80K was detected by both DS and PS in 43/61 (70.5%) genotype 1a patients at a median DS read frequency of 99.6%. Three additional patients (including 1 genotype 1b patient) had Q80K detectable by DS only (read frequency range: 4.6–13.0%); all 3 patients achieved SVR12. One additional patient had minority NS3 baseline polymorphism known to affect *in vitro* SMV activity (D168N at a read frequency of 1.3%) and achieved SVR12. The emerging NS3 mutations observed at time of relapse by PS were not detected at baseline or early on-treatment by DS. No additional emerging NS3 minority mutations were observed at time of relapse by DS. No emerging NS5B mutations were observed by PS or DS.

Conclusions: Relapse in the COSMOS study was not associated with the presence of minority baseline polymorphism and/or early on-treatment emergence of NS3/NS5B minority mutations. No SOF resistance mutations were observed at time of relapse with DS.

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P0781

HIGH SVR RATES DESPITE MULTIPLE NEGATIVE PREDICTORS IN GENOTYPE 1 PATIENTS RECEIVING OMBITASVIR/PARITAPREVIR/R, DASABUVIR WITH OR WITHOUT RIBAVIRIN FOR 12 AND 24 WEEKS: INTEGRATED ANALYSIS OF SIX PHASE 3 TRIALS

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Background and Aims: Phase 3 studies of the 3 direct-acting antiviral (3D) regimen of ombitasvir co-formulated with

paritaprevir/ritonavir (PTV/r, identified by AbbVie and Enanta) and dasabuvir with or without ribavirin (RBV) have demonstrated high efficacy in HCV genotype 1-infected patients regardless of host, viral, and disease characteristics. Whether multiple negative predictors of response have meaningful impact on SVR rates was evaluated using data from six phase 3 studies.

Methods: Multivariate stepwise logistic regression was performed on >2000 HCV genotype 1-infected patients treated with 3D +/− RBV in the SAPPHIRE-I, SAPPHIRE-II, TURQUOISE-II, PEARL-II, PEARL-III, and PEARL-IV studies to identify factors associated with reduced SVR12 rates. Independent continuous variables included age, baseline BMI, weight, HCV RNA, and alanine aminotransferase level; independent categorical variables included HCV subgenotype, IL28B genotype, sex, race, ethnicity, geographic region, fibrosis stage, cirrhosis, treatment regimen (3D vs 3D + RBV), treatment duration (12 vs 24 weeks for patients with cirrhosis), prior HCV treatment experience, and history of diabetes, depression or bipolar disorder, bleeding disorders, and former injection drug use (IDU). Significance level for entering and exiting the model was 0.1.

Results: Five independent variables were associated with lower SVR12 rates: HCV genotype 1a, higher baseline weight, IL28B TT genotype, Hispanic/Latino ethnicity, and higher baseline HCV RNA. The overall SVR12 rate was 96.2% (1964/2041); SVR12 rates were 99% in patients with 0 or 1 negative predictor, and 97% and 95% in those with 2 or 3 negative factors, respectively. SVR rates declined to <95% only among patients with a combination of 4 or 5 negative predictors of response, 86% (106/123) and 82% (9/11) in patients with 4 or 5 negative predictors, respectively.

Conclusions: The 3D +/− RBV regimen achieves high SVR rates across patient populations with multiple negative predictors. SVR12 rates among patients with multiple negative factors using approved treatment regimens will be presented.

P0782

ALL-ORAL 12-WEEK COMBINATION TREATMENT WITH DACLATASVIR (DCV) AND SOFOSBUVIR (SOF) IN TREATMENT-EXPERIENCED PATIENTS INFECTED WITH HCV GENOTYPE (GT) 3: A SUBANALYSIS OF THE ALLY-3 PHASE 3 STUDY

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Background and Aims: The phase 3 ALLY-3 study evaluated the all-oral, ribavirin (RBV)-free combination of daclatasvir (DCV; pangenotypic NS5A inhibitor) and sofosbuvir (SOF; NS5B polymerase inhibitor) in patients with GT3 infection. After 12 weeks of treatment, sustained virologic response at posttreatment Week 12 (SVR12) was achieved by 90% and 86% of treatment-naive and -experienced patients, respectively.

Methods: Treatment-naive (N = 101) and experienced (N = 51) patients received open-label DCV 60 mg + SOF 400 mg once daily for 12 weeks. This subanalysis provides further details of efficacy and safety outcomes in the experienced cohort.

Results: Treatment-experienced patients were predominantly male (63%), white (88%) and non-cirrhotic (67%); 75% had baseline HCV RNA ≥800K IU/mL, and 61% had non-CC *IL28B* genotypes. Patients had previously received IFN-based (n = 42), SOF-containing (n = 7, including 7 treated with SOF/RBV and 1 who was retreated with SOF/peg/RBV) and alisporivir-containing (n = 2) regimens. Prior responses included relapse (n = 31), null response (n = 7), partial response (n = 2), and other forms of nonresponse or IFN intolerance (n = 11). All patients completed 12 weeks of study treatment. SVR12 was achieved in 44 patients (86%); all prior null and partial responders, and IFN-intolerant patients achieved SVR12. SVR12 rates were higher in patients without cirrhosis and in those with *IL28B* CC genotype (Table). Treatment failure (relapse) was experienced by 7 patients, including 5 prior IFN/RBV recipients (prior response: relapsers, n = 4; HCV RNA never undetectable, n = 1) and 2 patients who relapsed after prior treatment with SOF/RBV. Of the two prior SOF relapsers, one had cirrhosis with grade 1 steatosis, and one had Fibrotest F3 with grade 2 steatosis, and a baseline NS5A-Y93 resistance-associated variant. There were no serious AEs or AEs leading to discontinuation. Grade 3/4 AEs (a single report of arthralgia) and grade 3/4 lab abnormalities (platelets, n = 1; lipase, n = 1) were uncommon. The most frequent AEs (any grade) were fatigue (26%), headache (20%), nausea (14%) and arthralgia (12%). Safety parameters were similar in those with or without cirrhosis.

Parameter	SVR12, % (n/N)
All treatment-experienced	86 (44/51)
Prior IFN-based regimen	88 (37/42)
Prior sofosbuvir-containing regimen	71 (5/7)
Prior alisporivir-containing regimen	100 (2/2)
Patients without cirrhosis	94 (32/34)
Patients with cirrhosis	69 (9/13)
Fibrotest F0–3	91 (39/43)
Fibrotest F4	63 (5/8)
NS5A-Y93 baseline resistance-associated variant present	71 (5/7)
NS5A-Y93 baseline resistance-associated variant absent	88 (38/43)
IL28B CC	95 (19/20)
IL28B non-CC	81 (25/31)

Conclusions: This all-oral, 12-week combination of DCV+SOF achieved high SVR12 rates in GT3 patients previously treated with all oral DAA or IFN-containing regimens. DCV+SOF was well tolerated.

P0783

VIRAL KINETICS DURING INTERFERON-FREE SOFOSBUVIR CONTAINING TREATMENT REGIMENS IN A REAL-LIFE COHORT OF CHRONIC HEPATITIS C PATIENTS WITH ADVANCED LIVER DISEASE

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Background and Aims: Sofosbuvir (SOF), Simeprevir (SMV) and Daclatasvir (DCV) are reimbursed for chronic hepatitis C patients with advanced liver disease (F3, F4) and posttransplant patients in Austria. Real-life data in these patients are scarce and the optimal duration of treatment is still under debate. The aim of this study was to evaluate viral kinetics in patients with advanced liver disease receiving interferon-free treatment regimens.

Methods: We included 181 patients (mean age: 55.8±10.1 years, m/f: 117/64, treatment experienced: 109 [60%], prior failure to protease inhibitors: 23 [13%]) with advanced fibrosis (Metavir score F3: n = 13, F4: n = 168; median platelet count: 98 G/l) who were treated with Sofosbuvir (SOF) 400 mg/day combined with 60 mg/day Daclatasvir (DCV) (GT 1, 3, 4; n = 86) or with Simeprevir (SMV) 150 mg/d (GT1 and 4; n = 49) or weight based ribavirin (RBV; GT 1–4; n = 46). Duration of treatment was at the discretion of the investigator. Viral load was measured after 48 hours, at weeks 1, 2, 3, 4 and then every 4 weeks until the end of treatment by Abbott RealTime HCV quantitative assay (lower limit of quantification [LLOQ]: 12 IU/ml) or by Roche COBAS AmpliPrep/ COBAS TaqMan HCV quantitative assay, Version 2 (LLOQ: 15 IU/ml).

Results: HCV-RNA dropped by a mean log of 2.91 and 3.54 after 48 hours and 7 days, respectively. At weeks 4, 8, 12, and 16 of treatment, 23%, 60%, 84%, and 93% of patients with advanced liver disease had undetectable HCV RNA (TND, target not detected), respectively. Currently 54 patients have reached end of treatment after a treatment duration of 12 weeks (n = 16), 16 weeks (n = 2) or 24 weeks (n = 36). 5 patients discontinued therapy due to psychiatric adverse events (n = 2), death (n = 2, sepsis and multiple organ failure due to decompensation), and non-adherence (n = 1). 29 patients reached follow-up week 4 (23 SVR4, 6 relapsed) and 4 reached follow-up week 12 (all SVR12). All relapsers received SOF/RBV treatment.

Conclusions: Determination of on-treatment response may help to optimize treatment regimen in patients with advanced liver disease. Patients with detectable HCV RNA at treatment week 12 (16%) may need a treatment of 24 weeks.

P0784

SOFOSBUVIR IN COMBINATION WITH PEGINTERFERON AND RIBAVIRIN FOR PATIENTS CHRONICALLY INFECTED WITH HEPATITIS C VIRUS GENOTYPE 4: "REAL-LIFE" EXPERIENCE OF TWO LARGE VIRAL HEPATITIS CENTERS IN NORTHERN GERMANY

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Background and Aims: Sofosbuvir (SOF)-based triple therapy has been approved for all hepatitis C virus (HCV) genotypes. However, real life efficacy data of SOF in patients with genotypes 4 to 6 are limited. Furthermore, SOF-based triple therapy was never directly compared to dual therapy with peginterferon (IFN) and ribavirin (RBV).

Methods: Every patient with HCV genotype 4, 5 or 6 infection who received IFN/RBV/SOF between January and June 2014 at our two centers was included, and patients' data were compared with historical HCV genotype 4 patients treated with IFN/RBV from 01/2001 to 12/2007.

	IFN/RBV/SOF N (%) Mean (±SD) Median (range)	IFN/RBV N (%) Mean (±SD) Median (range)	P-value
Male sex	21 (80.8%)	41 (83.7%)	0.757
Age [years]	47.00 (± 11.02)	42.33 (± 9.29)	0.072
Stage of fibrosis [METAVIR]			0.073
F0–2	13 (50%)	36 (73.5%)	
F3–4	13 (50%)	13 (26.5%)	
Cirrhosis (F4)	6 (23.1%)	6 (12.2%)	0.321
Treatment naïve	13 (50%)	39 (79.6%)	**0.017***
Treatment experienced			
Relapse	7 (26.9%)	3 (6.1%)	**0.017***
Non-response	5 (19.2%)	6 (12.2%)	0.498
Breakthrough	1 (3.8%)	1 (2.0%)	1
Baseline laboratory			
Viral load [U/mL]	645×10^3 (10^2 to 7×10^6)	70×10^3 (0.5×10^3 to 2×10^6)	**<0.001***
Haemoglobin [g/dL]	14.9 (11.8–17.2)	15.2 (11.5–18.3)	0.691
Platelets [billion/L]	200.5 (104–350)	220 (59–392)	0.589
Leucocytes [billion/L]	6 (2.5–10.6)	6.5 (2.9–15.6)	0.853
ALT [U/L]	56 (25–290)	63 (11–346)	0.997
Ribavirin dose reduction	7 (26.9%)	14 (28.6%)	1
Peginterferon dose reduction	1 (3.8%)	11 (22.4%)	**0.048***
RVR (N = 66)	17/24 (70.1%)	16/42 (38.1%)	**0.020***
EOTR	25/26 (96.2%)	30/49 (61.2%)	**0.002***
SVR12	19/22 (86.4%)	21/49 (42.9%)	**0.001***

Results: We included 75 patients in our study. 26 patients received SOF-based triple therapy and 49 patients were treated with IFN/RBV. Baseline characteristics of both groups are provided in the accompanying table. Sustained virological response (SVR12) was achieved in 19/22 (86.4%) patients who received IFN/RBV/SOF and in 21/49 patients (42.9%) of the control group (P = 0.001). Rapid virological response (RVR) was a predictor of SVR12 in the control group (13/16 with RVR compared to 4/26 patients without RVR achieved SVR12; P < 0.001), whilst patients with SOF-based therapy achieved SVR12 regardless of reaching RVR (SVR12 in 6/6 patients with RVR and in 13/15 patients without RVR; P = 1). All cirrhotic patients with SOF-based triple therapy achieved SVR12. Notably, adverse events (AEs) of any kind more frequently occurred in the control group than in the SOF group (95.9% vs. 69.2%; P = 0.002). Particularly gastrointestinal (P < 0.001), dermatologic (P = 0.003) and psychiatric (P = 0.030) AEs were more frequent in IFN/RBV patients than in patients receiving IFN/RBV/SOF. SVR data of 4 patients are pending, but will be provided in April 2015.

Conclusions: A 12 week course of IFN/RBV/SOF was significantly more effective than dual therapy for 48 weeks. Furthermore,

patients who received dual therapy displayed a higher risk to experience side-effects than patients with SOF-based triple therapy. Combination of sofosbuvir, peginterferon and ribavirin should be considered as first-line therapy for patients chronically infected with HCV genotypes 4 to 6, if all-oral therapy is not available.

P0785

FIRST REAL CLINICAL PRACTICE DATA IN SPAIN ON SOFOSBUVIR, SIMEPREVIR AND DACLATASVIR IN POST-TRANSPLANT HCV RECURRENCE: THE HepatiC REGISTRY EXPERIENCE

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Background and Aims: Direct-acting antivirals (DAA) have become elective treatment for HCV chronic infection. After phase III trials, sofosbuvir (SOF), simeprevir (SIM) and daclatasvir (DCV) have recently started their use in Spain in different combinations. The aim of this study is to analyse the initial experience in Spain on these new treatments in liver transplant (LT) patients.
Methods: AEEH's HepatiC is a multicentric monitored registry including HCV-infected patients under DAA. We selected 106 LT patients with post-LT HCV-recurrence under compassionate use. The main basal characteristics and safety data regarding SVR at week 4 post-end of treatment (SVR4) were assessed.
Results: Eighty-nine percent of the patients had G1 and the others G3–4. Cholestatic recurrence was present in 30% of patients and 34% had F4 fibrosis stage. At treatment onset, 26% had ascites (median MELD 12, 6–31). The most part of the patients (69%) were treatment-experienced, including new DAA (5%). Treatment groups: SOF/PR (n = 16), SOF/R (n = 52), SOF/DCV±R (n = 28), SIM/DCV±R (n = 10). Currently, 46/106 patients have data on SVR4. With complete data pending, global SVR is 80%. SVR4 by groups was: SOF/PR, 89% (8/9); SOF/R, 72% (21/29); SOF/DCV±R, 100% (6/6); SIM/DCV±R, 100% (1/1). No difference in SVR4 was found for patients with cholestatic recurrence (88.2% *vs* 71%, p = 0.284). Among patients with F4, those with liver decompensation had lower SVR4 than compensated ones (56% *vs* 88%, p < 0.05). Albumin and bilirubin levels significantly improved after treatment (Alb, 3.4 *vs* 4 g/dL, p < 0.001; Bil, 1.8 *vs* 1 mg/dL, p < 0.001), unlike platelets (88 *vs* 107×10^9/L, p = 0.207). Regarding safety, 36% of the patients experienced severe adverse events (SAE) during treatment, mainly transfusion-requiring anemia (22/38, 58%). Patients on ribavirin had more need for transfusion (24% *vs* 5%, p < 0.05). No association was observed between interferon use and SAE (38% *vs* 36%, p = 0.785), yet this group is small (n = 16). During treatment, 8/106 (8%) cases of grade 3–4 infection were registered. Nevertheless, only 7/106 (7%) patients required early discontinuation due to SAE.
Conclusions: First data on real clinical practice show that new DAA induce high RVS4 and improve hepatic function in a difficult-to-treat population as LT recipients. While SAE are still present, DAA discontinuation was necessary in a low proportion of patients. Complete data on SVR12 will be presented, including SOF+DCV and SIM+DCV with and without R, 12 vs 24 weeks.

P0786

100% SVR12 WITH LEDIPASVIR/SOFOSBUVIR±RIBAVIRIN FOR 12 WEEKS IN JAPANESE PATIENTS WITH CHRONIC GENOTYPE 1 HEPATITIS C VIRUS WHO PREVIOUSLY FAILED THERAPY WITH PROTEASE INHIBITOR + PEGYLATED INTERFERONα + RIBAVIRIN

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Background and Aims: Patients with HCV who have previously failed other therapies have lower rates of SVR12 than treatment naïve patients. This open-label, Phase 3 study evaluated the efficacy and safety of ledipasvir 90 mg/sofosbuvir 400 mg Fixed-Dose Combination (LDV/SOF FDC) ± ribavirin (RBV) administered orally, once daily for 12 weeks in Japanese patients with HCV GT1.
Methods: Patients were included if: age ≥20 years; HCV-RNA ≥10^5 IU/mL; platelets ≥50,000/μL. Primary endpoint was Sustained Virologic Response 12 weeks after treatment completion (SVR12). Here we present a post-hoc subgroup analysis of patients who failed previous therapy with a protease inhibitor (PI) plus pegylated interferon (Peg-IFNα) and RBV.
Results: Out of the total study population of 341 patients, 40 (12%) had previously failed treatment with PI+Peg-IFNα+RBV. In this subpopulation, the mean age was 60 years and 40% were male. In terms of their HCV disease characteristics, 93% were HCV GT1b, 25% were IL28 CC, 18% had cirrhosis and mean HCV RNA was 6.5 log10 IU/mL. Prior PI treatment included telaprevir (n = 14), simeprevir (n = 13), vaniprevir (n = 8), and faldaprevir (n = 5). Most (31/40) had previously relapsed, five were non-responders, and four were IFN intolerant.
All patients who had previously failed PI+ Peg-IFNα+RBV therapy (40/40) achieved SVR12 after 12 weeks of treatment with LDV/SOF±RBV. LDV/SOF±RBV was also safe to use in this population. In the LDV/SOF group, 8/17 (47%) patients reported a treatment-emergent adverse event (TEAE). All of these were mild and only one was considered related to study drug. In the

LDV/SOF+RBV group, 18/23 (78%) reported a TEAE. All were mild to moderate in severity and most were considered related to study drug. The most common AE in both arms was nasopharyngitis. In the LDV/SOF+RBV arm, RBV-related AEs such as anemia, nausea and rash were also reported. There were no Grade 3 or 4 AEs nor discontinuations due to AE in either arm.

Conclusions: LDV/SOF FDC±RBV for 12 weeks achieved SVR12 in 100% of Japanese patients who had previously failed PI+ Peg-IFNα+RBV. LDV/SOF FDC for 12 weeks provides a highly effective, well-tolerated, IFN- and RBV-free treatment for Japanese patients with chronic HCV GT1 infection.

P0787

COMPARISON OF SOFOSBUVIR +/− SIMEPREVIR IN HETEROGENEOUS, REAL-WORLD POPULATIONS OF HCV PATIENTS OVER 70 YEARS OF AGE VS YOUNGER HCV PATIENTS; DATA FROM THE TRIO NETWORK

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Background and Aims: Clinical trials often exclude significant numbers of patients over the age of 65 years and data on treatment of older patients with HCV is lacking. Trio Health is a disease management company that works in partnership with academic medical centres, community physicians and specialty pharmacies in the US to optimize care for Hepatitis C. Data obtained through the Trio Health program were used to evaluate efficacy and tolerability of regimens containing sofosbuvir or simeprevir in HCV patients over 70 years of age in comparison to HCV patients 70 years or younger.

Methods: Data were collected from Rx records through the Trio Platform in partnership with specialty pharmacies. Analyses were limited to 955 patients who initiated treatment with 12 week regimens between December 2013 and March 2014. Patients were treated in 142 clinics, 30 of which were academic-based. 55 of the 955 patients were over 70 years of age.

Table 1. Patient demographics and clinical characteristics at baseline

	Age ≤70 years, N = 900 (94%)	Age >70 years, N = 55 (6%)
Age, mean (range)	56 (17–70)	74 (71–86)
Male, no. (%)	535 (59%)	30 (55%)
Black, no. (%)	141 (16%)	10 (18%)
Platelets <100K/ul, no. (%)	122 (16%)	10 (20%)
Treatment experienced, no. (%)	392 (44%)	15 (27%)
Cirrhosis, no. (%)	268 (30%)	23 (42%)
Genotype, no. (%)		
1	61 (7%)	1 (2%)
1a	448 (50%)	14 (25%)
1b	159 (18%)	20 (36%)
2	194 (22%)	18 (33%)
3	7 (1%)	0 (0%)
4–6	24 (3%)	1 (2%)
mixed	7 (1%)	1 (2%)
Initial viral load, no. (%)		
≤800,000 IU/ml	283 (31%)	26 (47%)
>6MM IU/ml	169 (19%)	9 (16%)
Regimen, no. (%)[a]		
PEG + RBV + SOF	377 (42%)	7 (13%)
RBV + SOF	207 (23%)	20 (36%)
SMV + SOF +/− RBV	292 (32%)	28 (51%)

[a] 24 patients received other therapies.

Results: Patient demographics and baseline clinical measures are provided in Table 1. In the over 70 years old group, the discontinuation rate was 7% and SVR12 rates were 79% and 95% for the Intent to Treat (ITT) and Per Protocol (PP) populations, respectively, with results pending for 8 patients. In comparison and without balancing for disease, treatment and/or patient characteristics, outcomes for ≤70 year old patients (n = 900) from the same time period were 4% discontinuation, 80% SVR12 (ITT) and 87% SVR12 (PP), with results pending for 119 patients. None of the rates were significantly different between the age groups, though the SVR12 PP rate difference approached significance at p = 0.056.

Conclusions: All oral DAA therapy is well tolerated in patients over 70 years old with SVR rates >80% similar to that seen in younger populations.

P0788

ESTIMATING THE COST-EFFECTIVENESS OF DACLATASVIR PLUS SOFOSBUVIR IN TREATMENT-NAÏVE, TREATMENT-EXPERIENCED AND INTERFERON-INELIGIBLE/INTOLERANT PATIENTS WITH ADVANCED CHRONIC HEPATITIS C GENOTYPE 1 INFECTION

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Background and Aims: The availability of effective treatment for chronic hepatitis C patients with advanced disease has been considered an area of unmet medical need due to the limited efficacy and sub-optimal safety of historical treatments. However, the treatment landscape for chronic hepatitis C is rapidly evolving. Presented herein is a cost-effectiveness analysis of licensed regimens for treatment-naïve, treatment-experienced and interferon-ineligible/intolerant patients infected with hepatitis C virus (HCV) genotype 1 with advanced disease (METAVIR score ≥F3).

Methods: A published Markov model was used to estimate the relative cost-effectiveness of chronic hepatitis C treatments over a lifetime horizon in a cohort that had a mean age of 50 years and were 67% male. Patients progress through disease stages using published transition rates. Patients were distributed across fibrosis stages F3 and F4 at therapy initiation according to UK data (78.62% and 21.38%, respectively). Disease state costs were obtained from 2013 UK data and discounting was set to 3.5%. Weekly treatment costs were as follows: daclatasvir (DCV): £2,083.13; sofosbuvir (SOF): £2,915.24; simeprevir (SMV): £1,866.50; telaprevir (TVR): £1,866.50; boceprevir (BOC): £700.00; ribavirin (RBV): £66.95;

Population	Regimen	SVR (%)	ICER (£) versus DCV+SOF
Naive	DCV+SOF	100	
	TVR+PR	66	7,851
	BOC+PR	41	1,346
	SOF+PR	80	14,240
	SMV+PR	68	12,265
	PR	41	8,861
	No treatment	0	4,263
Experienced	DCV+SOF	100	–
	No treatment	0	4,263
Interferon-ineligible/intolerant	DCV+SOF	100	–
	SOF+RBV	36	Dominant[a]
	SMV+SOF	93	Dominant[a]
	No treatment	0	4,263

[a] DCV+SOF is associated with improved quality of life and reduced total cost.

pegylated interferon-alfa+ribavirin (PR): £191.35. Clinical inputs were obtained from pivotal studies for all treatment regimens, as reported in respective Summaries of Product Characteristics (SPCs) or published articles.
Results: Predicted incremental cost-effectiveness results are presented in Table 1.
Conclusions: Treatment with DCV+SOF is likely to be cost-effective (at the £20,000/QALY threshold) and associated with improved quality of life against all available comparators in treatment-naïve, treatment-experienced and interferon-ineligible/intolerant HCV genotype 1 patients with advanced disease; further, DCV+SOF is expected to be associated with reduced total medical costs in interferon-ineligible/intolerant patients versus active comparators.

P0789
EFFECTIVENESS, SAFETY AND COST PER SVR IN GT4 HEPATITIS C PATIENTS TREATED WITH SOFOSBUVIR-BASED THERAPIES IN REAL CLINICAL PRACTICE: A FRANCO-GERMAN EXPERIENCE (SOFEX-4)

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Background and Aims: Sofosbuvir (SOF) is approved for treatment of chronic hepatitis C (CHC) patients genotypes (GT) 1–6 but clinical trial data with SOF regimens in GT4 is limited. In the real-world (RW) setting, very limited clinical data was recently presented (Buggisch et al. AASLD 2014 n = 18, TRIO AASLD 2014 n = 16), and no study to date has simultaneously evaluated the clinical and economic outcomes of SOF-based therapies in GT4 patients in Europe. The aim of this study was to estimate the total costs, RW effectiveness, safety and cost per SVR of CHC patients treated with SOF-based therapies in a Franco-German collaboration.
Methods: This is a retrospective multicentre cohort study including 160 GT4 patients treated in 5 centers (3 in France and 2 in Germany). All patients who received at least 1 week of SOF from January 17th 2014 (date of SOF approval by EMA) to October 31st 2014, are included. SVR rates (ITT), overall and by subgroup, along with incidence of adverse events (AE) requiring intervention, were estimated. Total cost incurred from therapy initiation to 12 weeks after treatment was estimated by summing drug costs, lab costs, patient care costs including AE (treatment related only) management costs. Cost per SVR was calculated as median cost divided by the SVR rate. Subgroup analysis included treatment experience and fibrosis stage.
Results: Complete results will be available at the time of presentation. Recruitment is on-going and preliminary results are based on 51 patients with 49% receiving SOF+PR, 33% SOF+SIM, 10% SOF+R and 8% SOF+DCV. Mean age was 54 years, and 88% were male. At study entry, 57% of patients were cirrhotic and 73% had received at least one prior therapy (from the 31 patients with data available, 61% did not respond to prior therapy and 29% relapsed). Overall the SVR rate was 92% with 86% in treatment-naïve patients and 94% in treatment-experienced patients. A total of 11 patients (22%) experienced AEs (7 patients on SOF+PR). One patient discontinued due to AE. Cost per SVR results, overall and per subgroup will be estimated once patients have completed treatment and SVR12 is available.
Conclusions: Preliminary data indicates high levels of SVR in this difficult to treat mostly cirrhotic and experienced GT4 patients.

P0790
THE EFFECT OF RENAL IMPAIRMENT ON MULTIPLE-DOSE PHARMACOKINETICS OF THE FIXED-DOSE COMBINATION OF DACLATASVIR/ASUNAPREVIR/BECLABUVIR

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Background and Aims: Daclatasvir (DCV; NS5A inhibitor), asunaprevir (ASV; NS3 inhibitor), and beclabuvir (BCV [BMS-791325]; nonnucleoside NS5B inhibitor) are in phase 3 evaluation for chronic hepatitis C as a fixed-dose combination (FDC) of DCV/ASV/BCV (30/200/75 mg BID). As a subset of HCV patients have some degree of renal impairment due to age and comorbidities, this open-label, multiple-dose study (AI443–110) assessed the pharmacokinetics (PK) and safety of DCV/ASV/BCV FDC in HCV-uninfected subjects with normal renal function and renal impairment (RI) of varying degree.
Methods: All subjects were scheduled to receive DCV/ASV/BCV FDC + additional 75 mg BCV (to adjust for higher BCV exposures in HCV-infected patients) BID with food. Subjects were grouped based on renal function: normal renal function (creatinine clearance [CrCl] by Cockcroft-Gault of ≥90 mL/min), mild RI (CrCl 60 to <90 mL/min), moderate RI (CrCl 30 to <60 mL/min), severe RI (CrCl <30 mL/min) and end-stage renal dysfunction (ESRD) on hemodialysis (HD). All subjects received the regimen for 9 days with a single morning dose on Day 10. Noncompartmental PK parameters were derived and linear regression was used to estimate the relationship between Cmax (ng/mL) and AUC_{TAU} (ng·.h/mL) and CrCl. Predicted values for each PK parameter and associated 90% CIs were presented for CrCl equal to 15, 30, 60, and 90 mL/min. Safety was assessed and reported.
Results: A total of 41 subjects received study drug across normal renal function (n = 8), mild RI (n = 9), moderate RI (n = 8), severe RI (n = 8), and ESRD on HD (n = 8) groups. Values of PK parameters relative to the normal renal function group predicted from the regression analysis of CrCl are shown in the table. Regression analysis indicated that Cmax and AUC_{TAU} increased with decreasing CrCl for all 3 drugs. For subjects with ESRD on HD, exposures were similar to subjects with normal renal function. Multiple doses of study drug were generally well tolerated in all subjects. There were no deaths or SAEs. There was 1 discontinuation due to an AE (in moderate RI group) due to increased blood uric acid in a subject with baseline abnormalities, which was assessed as moderate and study drug-related.
Conclusions: No dose adjustment is recommended for renal impairment except for severe RI subjects that are not on HD, where QD dosing of the FDC is recommended instead of BID dosing.

Group	Adjusted Geometric Mean Ratio (90% CI) (vs Normal)						Dose adjustment
	DCV		ASV		BCV		
	Cmax	AUC_{TAU}	Cmax	AUC_{TAU}	Cmax	AUC_{TAU}	
Mild [a]	1.16 (1.03, 1.31)	1.22 (1.09, 1.37)	1.29 (0.98, 1.69)	1.33 (1.12, 1.59)	1.15 (1.02, 1.29)	1.28 (1.14, 1.45)	No
Moderate [a]	1.35 (1.19, 1.52)	1.50 (1.33, 1.68)	1.65 (1.26, 2.17)	1.76 (1.47, 2.11)	1.32 (1.17, 1.49)	1.65 (1.45, 1.86)	No
Severe [a]	1.45 (1.29, 1.63)	1.65 (1.47, 1.86)	1.88 (1.43, 2.47)	2.03 (1.69, 2.43)	1.42 (1.26, 1.60)	1.86 (1.65, 2.11)	QD instead of BID
ESRD [b]	0.95 (0.72, 1.25)	1.00 (0.76, 1.33)	0.89 (0.45, 1.73)	0.84 (0.52, 1.35)	0.93 (0.70, 1.23)	1.03 (0.75, 1.40)	No

[a] Estimated by linear regression analysis.
[b] Estimated by categorical analysis.

P0791

DACLATASVIR (DCV) COMBINED WITH SOFOSBUVIR (SOF) OR SIMEPREVIR (SMV) IN LIVER TRANSPLANT (LT) RECIPIENTS WITH SEVERE RECURRENT HCV GENOTYPE 1 INFECTION

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Background and Aims: Interferon-based therapies are poorly tolerated in LT recipients with recurrent HCV. DCV, a potent NS5A inhibitor, has shown promising efficacy when combined with SOF or SMV + ribavirin (RBV). The aim of this study was to describe the results of an ongoing study of DCV-based therapy in LT recipients.
Methods: DCV 60 mg/day was administered for up to 24 weeks as part of a compassionate use protocol. Safety and efficacy laboratory results, as well as clinical data, were obtained from local site investigators.
Results: As of 22/11/14, 108 patients (pts) had enrolled, and end of treatment (EOT) data were available for 64 eligible pts. The mean time from LT to initiation of DCV was 42.6±56.9 months (33% <1 year). Mean age was 59.3±8.8 years, 66% were male, 44% had fibrosing cholestatic HCV, 30% had cirrhosis, 8% had HIV co-infection, and 67% had received prior antiviral therapy. The % CTP A/B/C score was 52%/27%/16%; mean MELD score was 12.8±6.3. Most pts had HCV genotype 1 (34% 1a, 59% 1b). Mean baseline HCV RNA was $18\times6\log_{10}$ IU/mL. Oral antiviral regimens included DCV+SOF (n = 48), DCV + SMV (n = 14), and DCV+SMV+SOF (n = 2), and 34% overall received RBV. Five pts died between 0 and 16 weeks of DCV therapy from liver failure or sepsis not attributed to DCV (all in the DCV + SOF group). In the 59 survivors, 3 pts (5%) had virologic breakthrough at weeks 12, 16, and 21 after DCV start, and 2 EOT responder pts (3%) had a virologic relapse 5 and 13 weeks after EOT (all 5 pts received DCV + SMV ± RBV). Twenty pts were observed ≥12 weeks after EOT; at the first measurement ≥12 weeks, 85% of these 20 pts were HCV-RNA (−), 10% were HCV-RNA (+) but <43 IU/mL, and 1 pt had 15,400 IU/mL; the second pt who relapsed had 13,510 IU/mL at the last measurement at week 5 post-EOT. The mean CTP and MELD scores improved from baseline to last follow-up (CTP: 6.9 vs 5.7, $P < 0.0001$, MELD: 11.9 vs 10.0, $P = 0.0006$) in 50 (CTP) and 52 (MELD) evaluable pts with a median follow-up of 29 weeks.
Conclusions: A 24-week course of DCV-based oral antiviral therapy was well tolerated and resulted in a high EOT virologic response rate in LT recipients with severe recurrent HCV. The majority of DCV-treated pts experienced stabilisation or improvement in their laboratory results and clinical status. However, the optimal DCV-based all-oral antiviral combination regimen that will maximise the likelihood of SVR12 in LT recipients requires further study.

P0792

BASELINE FACTORS ASSOCIATED WITH INCREASED SVR RATES IN 123 TREATMENT-NAÏVE CHRONIC HCV GENOTYPE 1 PATIENTS TREATED WITH A SHORTENED 12-WEEK SIMEPREVIR PLUS PEGYLATED INTERFERON AND RIBAVIRIN REGIMEN: A MULTIVARIATE ANALYSIS

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Background and Aims: A Phase 3, open-label study of SMV (150 mg QD) + PR was conducted in Europe, in 163 treatment-naïve chronic HCV genotype 1 patients with mild-to-moderate fibrosis (F0–F2, Metavir score) to investigate safety and efficacy of a 12-week SMV+PR regimen. This pre-planned, multivariate analysis aimed to determine baseline factors associated with SVR12, and identify patients who may benefit from shorter interferon-based therapy.
Methods: Patients with HCV RNA <25 IU/mL (detectable/undetectable [using Roche COBAS® Taqman® assay, LLOQ: 25 IU/mL, LLOD: 15 IU/mL]) at Week 2, and <25 IU/mL undetectable at Week 4 and 8 fulfilled criteria for stopping all therapy at Week 12. If these criteria were not met, PR was continued to Week 24. Patients eligible to stop therapy at Week 12 were included in the univariate/multivariate analyses to determine factors influencing either SVR or relapse.
Results: Overall, 75% (123/163) of patients were eligible for 12 weeks of therapy. Of these, 65% (80/123) achieved SVR12; 39 patients relapsed and 4 did not have SVR12 data. SVR varied according to baseline parameters, e.g. 94% (30/32), 53% (39/73) and 61% (11/18) for *IL28B* CC, CT and TT, respectively, and 82% (27/33) and 59% (53/90) for HCV RNA ≤800,000 and >800,000 IU/mL, respectively. In univariate/multivariate analyses, baseline HCV RNA, *IL28B* CC genotype, and mild fibrosis (F0/F1) were associated with SVR and relapse (Table). Baseline HCV RNA and mild fibrosis were associated with SVR and relapse when considering the non-CC population (N = 91) (Table). HCV genotype subtype, race, sex and baseline BMI were not associated with SVR in either the full or non-CC patient populations. Although Week 2 viral response was associated with SVR in the univariate analysis of the full population (odds ratio 2.52 [1.13, 5.60], P = 0.0235) this was not the case in the multivariate analysis (odds ratio 1.92 [0.70, 5.29], P = 0.2068). Similar results were found for the association of Week 2 viral response with SVR in the non-CC population and with relapse in both populations analysed. The safety profile was similar to previous SMV+PR studies.
Conclusions: Early on-treatment response patients with *IL28B* CC genotype, mild fibrosis (F0/F1), and lower baseline HCV RNA levels are more likely to achieve SVR12 when stopping all therapy after 12 weeks of SMV+PR. In the non-CC patient population, mild fibrosis

and lower baseline HCV RNA levels were associated with SVR on this shortened regimen.

Table: Factors associated with SVR and viral relapse determined by univariate and multivariate analyses in patients eligible for 12 weeks of therapy

Baseline factor	N	Univariate analysis		Final multivariate analysis	
		Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
SVR, all patients					
Baseline viral load (log_{10} HCV RNA IU/mL)	123	0.53 (0.29, 0.95)	0.0339	0.24 (0.10, 0.57)	0.0012
IL28B genotype CC	123	12.3 (2.77, 54.5)	0.0010	31.1 (5.77, 168)	<0.0001
METAVIR fibrosis score F0–F1	122	4.45 (1.85, 10.7)	0.0009	6.96 (2.25, 21.5)	0.0007
SVR, non-CC patients					
Baseline viral load (log_{10} HCV RNA IU/mL)	91	0.19 (0.08, 0.46)	0.0002	0.17 (0.06, 0.43)	0.0002
METAVIR fibrosis score F0–F1	91	6.37 (2.09, 19.4)	0.0011	8.02 (2.23, 28.8)	0.0014
Relapse, all patients					
Baseline viral load (log_{10} HCV RNA IU/mL)	122	2.30 (1.21, 4.38)	0.0109	6.73 (2.50, 18.1)	0.0002
IL28B genotype CC	122	0.04 (0.01, 0.34)	0.0027	0.01 (0.00, 0.12)	0.0001
METAVIR fibrosis score F0–F1	121	0.22 (0.09, 0.54)	0.0008	0.12 (0.03, 0.40)	0.0007
Relapse, non-CC patients					
Baseline viral load (log_{10} HCV RNA IU/mL)	90	5.86 (2.32, 14.8)	0.0002	6.70 (2.48, 18.1)	0.0002
METAVIR fibrosis score F0–F1	90	0.18 (0.06, 0.52)	0.0016	0.14 (0.04, 0.49)	0.0021

P0793
ESTIMATING THE NUMBER OF CHRONIC HEPATITIS C PATIENTS IN NEED OF TREATMENT AND PROJECTING THE DISEASE BURDEN DURING 2015–2030 UNDER THE INTERFERON-FREE TREATMENT REGIMENS IN GREECE

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Background and Aims: The treatment of hepatitis C with IFN-free Direct-Acting Antivirals (DAAs) is anticipated to change the future burden of disease. Aim of this study is to estimate the current number of diagnosed patients in need of treatment and to assess the impact of IFN-free DAAs on HCV-related morbidity and mortality in Greece.
Methods: A previously described model has been used (Razavi et al, Hatzakis et al, J Viral Hepat 2014) to estimate the current number of patients in each fibrosis stage and to make projections for the future disease burden up to 2030. We used a previously published estimate that 19.2% of Greek HCV-infected patients have been diagnosed (Papatheodoridis et al, J Viral Hepat 2014) to calculate the number of diagnosed cases per fibrosis stage. We projected the future disease burden under: (A) Base scenario: treatment of approximately 2,000 patients/year (30–69 years old, independently of fibrosis stage) with Peg-interferon+Ribavirin with or without Boceprevir/Telaprevir, (B) IFN-free scenario: treatment of 3,000 patients/year for 2015 and 2016 and approximately 2000/year for the subsequent years with IFN-free DAAs (30–69 years old, F3–F4 only).
Results: The number of diagnosed patients with chronic hepatitis C in 2014 in Greece is estimated to be 25600. Of those, 2650, 2850, 4100, 7150, 6750 and 2100 are diagnosed with F0, F1, F2, F3, F4-compensated and F4-decompensated cirrhosis, respectively. Under the base scenario, no decline is predicted by 2030 in HCV-related morbidity and mortality. Under the IFN-free scenario, the number of patients with F4-compensated or F4-decompensated cirrhosis, HCC and the number of deaths in 2030 are predicted to be 34–46% lower than the base scenario. Furthermore, from 2024 and onwards, patients in earlier fibrosis stages could be treated due to the exhaustion of diagnosed F3–F4 cases. In total, 31460 and 30920 will have received treatment during 2015–2030 under the base and the IFN-free scenario, respectively.
Conclusions: Current guidelines suggest that treatment should be prioritized depending on the fibrosis stage. Estimates of the number of diagnosed patients per fibrosis stage are necessary for appropriate public health planning. In Greece, there are approximately 16000 diagnosed patients with fibrosis stage ≥F3 that should be prioritised for treatment. If those patients are given access to the new treatment regimens, a considerable decline is predicted in HCV-related morbidity and mortality by 2030.

P0794
RELATIONSHIP BETWEEN HCV GENOTYPE, LIVER CO-MORBIDITIES AND FIBROSIS IN THE FRENCH COHORT ANRS CO22 HEPATHER

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Background and Aims: ANRS CO22 HEPATHER is a French multicenter cohort (32 centres) aiming to include 15,000 HCV- and 10,000 HBV-infected patients. We analyzed the factors associated with a specific HCV genotype, especially the fibrosis stage and the liver comorbidities.
Methods: 14,698 patients were included on November 19th 2014; 10,197 had HCV hepatitis, 8519 had a chronic hepatitis including 4403 patients with complete data regarding the viral genotype, liver comorbidities and general characteristics at baseline. The association between the viral genotype, liver comorbidities and

fibrosis was assessed by multinomial logistic regression adjusted on age, gender, duration of diagnosis and treatment status at baseline; genotype 1-infected patients were considered as the reference category.

Results: Genotype 3- or 4-infected patients were younger and genotype 2- or 5-infected patients were older than genotype 1-infected patients. The proportion of men was higher in genotype 3 and 4 and lower in genotype 2 (Table). Treatment-naïve patients were more frequent in genotypes 2 and 5 and less frequent in genotypes 4. The viral load at baseline did not significantly differ between genotypes. Extensive fibrosis or cirrhosis (F3/4) were more frequent in genotypes 3 (60% and 42%, respectively) and less frequent in genotypes 2 (33% and 21%), and 5 (34% and 24%), than in genotypes 1 (51% and 34%). Hazardous alcohol consumption was 2-fold higher in genotype 3 and could be an additional risk factor for fibrosis, while metabolic comorbidities were less prevalent.

Table: General characteristics of the first 4403 HCV patients of the French HEPATHER ANRS CO22 cohort according to the genotype at inclusion.

Variable	Geno1 (ref) (n = 2919)	Geno2 (n = 245)	Geno3 (n = 577)	Geno4 (n = 571)	Geno5 (n = 91)
Age (yr)	58	**59**↑	**53**↓	**54**↓	**62**↑
Duration since diagnosis (yr)	14	**12**↓	13	**12**↓	12
Sex M (%)	55	**43**↓	**69**↑	**65**↑	45
Treatment-Naïve (%)	33	**52**↑	38	**33**↓	**53**↑
Viral load at inclusion (log_{10} IU/ml)	5.64	5.66	5.51	5.64	5.69
F3-F4 (%)	51	**33**↓	**60**↑	49	**34**↓
Suspicion of cirrhosis (%)	34	**21**↓	**42**↑	34	24
Obesity/overweight (%)	23	24	19	26	31
Diabetes (%)	14	14	11	15	15
Hypercholesterolemia (%)	7	10	**4**↓	6	**16**↑
Arterial hypertension (%)	30	33	**17**↓	**30**↑	37
Vasculitis with manifestations (%)	10	**4**↓	8	**6**↓	8
Renal failure (%)	3	**5**↑	2	**5**↑	5
Hazardous alcohol consumption (%)	3	3	**6**↑	4	2

A number in **bold** followed by ↓ (←) indicates a statistically significant **decrease** (**increase**) compared to genotype 1-infected patients.

Conclusions: HCV genotype3 chronic hepatitis is associated with more severe fibrosis than other genotypes.

P0795

SAFETY AND EFFICACY OF SOFOSBUVIR-CONTAINING REGIMENS IN THE FRENCH OBSERVATIONAL COHORT ANRS CO22 HEPATHER

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Background and Aims: The French cohort ANRS CO22 HEPATHER aims to identify prognosis factors, including response to treatments, in 10,000 HBV and 15,000 HCV-infected patients with a 8 years follow-up. In November 2014, 10,197 HCV patients from 32 centers were included. The aim of this analysis was to describe the profile of patients who started a Sofosbuvir (SOF)-containing regimen before May 25th, 2014 in the cohort and to evaluate the virological efficacy and safety of such regimens.

Methods: Demographics, history of liver disease were collected at entry in the cohort. Clinical, adverse events, and virological data were collected throughout treatment and post-treatment follow-up. Patients with prior liver transplantation, HCV therapy without SOF or participating in clinical trials were excluded.

Results: 619 patients in the cohort started a treatment with a SOF-containing regimen. Compared with 4523 patients who did not start a treatment; treated patients were older (57 vs 56 years), more frequently male (65% vs 54%), with a higher BMI (26 vs 25 kg/m^2), with a more advanced disease (cirrhosis 69% vs 14%; decompensated cirrhosis 5% vs 2%), more associated comorbidities (diabetes 19% vs 12%) and were more frequently treatment-experienced (previous PEG/RBV +/− 1st generation protease inhibitor 68% vs 30%). Regimens distribution according to HCV genotype is described in the table.

The baseline characteristics did not differ according to the regimen but decompensated cirrhosis was more frequent in patients starting a SOF/DCV +/− RBV regimen than in others (11% vs 2%). To date, the end-of-treatment response rates were >90% in all regimens, and the sustained virological response rates at week 4 were 77% with SOF/RBV, 79% with SOF/PEG/RBV, 89% with SOF/DCV, 100% with SOF/DCV/RBV, 95% with SOF/SMV and 100% with SOF/SMV/RBV; 78 SAE (7 of which considered treatment-related) were reported in 54 (8.5%) patients and 3 deaths (SOF/RBV: 1 hepatic failure, SOF/DCV: 1 cerebral haemorrhage and 1 septic shock). Only 20 patients (3%) have discontinued treatment prematurely (follow-up ongoing).

	HCV genotype			
	1	2	3	4
N	332	64	129	89
Treatment – n				
SOF/RBV	42	62	58	27
SOF/PEG/RBV	56	1	43	23
SOF/DCV	157	1	18	14
SOF/DCV/RBV	58	0	10	8
SOF/SMV	16	0	0	13
SOF/SMV/RBV	3	0	0	4

DCV: daclatasvir, SMV: simeprevir. 5 patients with genotype 5 infection not shown.

Conclusions: Sofosbuvir-containing regimens are highly efficient and fairly tolerated in "priority" patients. SVR12 and safety data will be reported for each regimen at the meeting.

P0796

NEW DIRECT ACTING ANTIVIRALS FOR HEPATITIS C TREATMENT: FIRST RESULTS IN A REAL-LIFE SETTING IN FRANCE

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Background and Aims: New direct antiviral agents (NDAAs) appear as a striking progress in hepatitis C treatment but have not largely been evaluated in real-life settings. The aim of this study was to evaluate the efficacy and safety in patients with chronic hepatitis C treated with NDAAs in general hospitals in France.

Methods: APROVVIE 2 is a longitudinal observational study of patients treated with NDAAs in French general hospitals (first in early access programs, then following market authorizations). Demographic, clinical, adverse events, biological and virological data are prospectively collected throughout treatment and post treatment follow-up from willing patients by their responsible physicians, voluntary investigators, members of the Association Nationale des Gastroentérologues des Hôpitaux généraux (ANGH).

Results: Since March, 2014, 287 patients with chronic hepatitis C starting treatment including NDAAs were included by 18 investigators in 16 centers. Main characteristics of the patients were: male gender 64%, median age 54 years, cirrhosis (C) 47%, F3 fibrosis stage 41%, high viremia 72%, patients naïve 29%, treatment-experienced 71% (non responders 39%, relapsers 32%), genotype (G) 1: 65%, genotype 2: 5%, genotype 3: 14%, genotype 4: 14%, genotype 5: 2%. Prescribed drugs were sofosbuvir (So) 97%, daclatasvir (Da) 49%, simeprevir (Si) 23%, peginterferon (IF) 18%, ribavirin (Riba) 32%.

G1 (n = 187): C 45%, Naive Pts 28%, IF-So-Riba 16%, IF-Si-Riba 3%, So-Si 20%, So-Da 56%, So-Riba 5%, HCV-RNA undetectable at week 4 (VR4) 77%.

G2 (n = 13): C 20%, Naive Pts 38%, IF-So-Riba 15%, So-Si 8%, So-Riba 77%, VR4 100%.

G3 (n = 40): C 62%, Naive Pts 30%, IF-So-Riba 15%, I So-Si 3%, So-Da 53%, So-Riba 30%, VR4 68%.

G4 (n = 40): C 41%, Naive Pts 23%, IF-So-Riba 13%, IF-Si-Riba 8%, So-Si 43%, So-Da 25%, So-Riba 13%, VR4 80%.

G5 (n = 7): C 60%, Naïve Pts 43%, IF-So-Riba 14%, So-Da 71%, So-Riba 14%, VR4 77%.

To date, on-treatment response (undetectable HCV-RNA) was observed in 42% and 76% of patients at week 2 and week 4, respectively. Grade 3–4 adverse effects have been so far reported in 6/52 patients receiving interferon-ribavrin, and 2/235 patients not receiving these drugs.

Conclusions: To date, patients treated in French general hospitals are mostly treatment-experienced ones with severe fibrosis/cirrhosis. Complete SVR and safety data will be presented.

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LATE RELAPSE IN PATIENTS WITH CHRONIC HEPATITIS C TREATED WITH TRIPLE THERAPY BASED ON FIRST GENERATION PROTEASE INHIBITORS

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Background and Aims: Sustained virological response 12 (SVR-12) or 24 (SVR-24) is the clinical event that defines chronic hepatitis C (CHC) cure and is considered equivalent to the eradication of the virus. Maintaining the response is the rule (>99%) and late relapses are exceptional. The aim was to analyze the incidence of late relapses in patients treated with first-generation protease inhibitors (PIs) [telaprevir (TVR) and boceprevir (BOC)] and to investigate whether relapse in patients with SVR-24 is due to re-infection or "real" late viral relapse.

Methods: We included 146 patients (54 HIV+) with CHC treated with first-generation PIs who had reached SVR-24 in our hospital. In patients with late relapse (after SVR-24) a phylogenetic analysis of the ENV gene of HCV by clonal sequencing (10 clones/sample) in baseline and relapse samples was performed. HCV-RNA was determined in peripheral blood mononuclear cells (PBMCs) samples.

Results: Only 2/92 (2.2%) HCV and 1/54 (1.8%) HCV/HIV patients experienced late relapse. The clinical features (fibrosis, genotype, treatment schedule) were: Case 1: F3; HCV-1b (12 weeks of triple therapy with PR/TVR and 24 weeks of double therapy with PR); Case 2: F2; HCV-1b (12 weeks of PR/PR and 12 of TVR); Case 3 (HIV+): F4; HCV-1a (4-weeks lead-in with PR and 44 of PR/BOC). All patients achieved extended rapid virological response. Relapse occurred between 6–15 months after SVR-24. Adherence to treatment was good in all cases. A high homology between baseline and relapse samples sequences was found (Case 1: 95%; Case 2: 95%; Case 3: 94%). Phylogenetic bootstrap analysis (90% significance) showed that baseline and relapse HCV-RNA sequences from each patient were always grouped together in a separate branch, showing a close relationship of both populations in all cases. Additionally, the complexity of baseline samples was always higher [different colonies (baseline vs relapse): 9/10 vs 2/10, 8/10 vs 2/10 and 10/10 vs 0/10 in cases 1, 2 and 3, respectively]. PBMCs samples (cases 1 and 2) taken at SVR-12 point were HCV-RNA positive.

Conclusions: These results confirm that late relapse, although a rare event, may occur in patients treated with first-generation PIs. Once re-infection is discarded, relapse may be due to a reactivation of residual viral strains, as demonstrated by sequence homology and low complexity of the emerging population. The persistence of HCV-RNA in PBMC may play a role in the relapse of these patients.

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THE COST-EFFECTIVENESS OF SOFOSBUVIR- AND SIMEPREVIR-BASED THERAPIES FOR HCV GENOTYPE 1 INFECTION

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Background and Aims: New regimens to treat HCV genotype 1 are highly effective, but costly. We estimated quality-adjusted life expectancy (QALE), costs, and incremental cost-effectiveness ratios (ICERs) for strategies to treating HCV genotype 1 treatment-naïve and treatment-experienced patients with and without cirrhosis.

Table: Cost-effectiveness of treatment regimens for HCV genotype 1 in treatment naïve and experienced patients with and without cirrhosis

Strategy	Cost	QALY	ICER [a]
Naïve No Cirrhosis, RNA <6 Million (8 weeks SOF/LDV)			
No treatment	161,000	8.5	–
PEG/RBV 48 weeks	202,000	11.0	dominated
PEG/RBV/SMV 24 weeks	227,000	13.8	dominated
SOF/LDV 8 weeks	229,000	14.3	11,800
PEG/RBV/SOF 12 weeks	260,000	14.1	dominated
Naïve No Cirrhosis, RNA ≥6 Million (12 weeks SOF/LDV)			
No treatment	161,000	8.5	–
PEG/RBV 48 weeks	202,000	11.1	dominated
PEG/RBV/SMV 24 weeks	227,000	13.8	12,400
SOF/LDV 12 weeks	257,000	14.3	63,900
PEG/RBV/SOF 12 weeks	260,000	14.1	dominated
Naïve Cirrhosis (12 weeks SOF/LDV)			
No treatment	96,000	4.8	–
PEG/RBV 48 weeks	156,000	7.0	dominated
PEG/RBV/SMV 24 weeks	201,000	10.9	dominated
PEG/RBV/SOF 12 weeks	248,000	12.3	dominated
SOF/LDV 12 weeks	252,000	13.9	17,000
Experienced No Cirrhosis (12 weeks SOF/LDV)			
No treatment	160,000	8.5	–
SOF/LDV 12 weeks	259,000	14.3	17,100
PEG/RBV/SOF 12 weeks	261,000	14.1	dominated
SOF/SMV 12 weeks	324,000	14.1	dominated
Experienced Cirrhosis (24 weeks SOF/LDV)			
No treatment	96,000	4.8	–
PEG/RBV/SOF 12 weeks	245,000	11.9	21,000
SOF/SMV 12 weeks	315,000	13.5	43,800
SOF/LDV 24 weeks	336,000	13.9	55,600

[a] dominated = a strategy that provides lower QALE at either higher cost, or a higher cost per QALY gained.

Methods: Monte Carlo simulation of HCV disease comparing current U.S. FDA approved regimens for HCV genotype 1 infection. We stratified patients by treatment experience, cirrhosis status and HCV RNA to model appropriate treatment course.
Treatment naïve patients (% SVR with/without cirrhosis, treatment course cost):
1. No treatment
2. Pegylated interferon (PEG) and ribavirin (RBV) 48 weeks (23/44%, $53,000)
3. PEG/RBV and simeprevir (SMV) 12 weeks (65/90%, $37,000)
4. PEG/RBV and sofosbuvir (SOF) 12 weeks (78/92%, $90,000)
5. SOF/ledipasvir (LDV) 8–12 weeks depending on cirrhosis and HCV RNA (96/96%, $58,000–87,000)

Treatment experienced patients:
1. No treatment
2. PEG/RBV/SOF 12 weeks (74/92%, $53,000)
3. SOF/SMV 12 weeks (91/93%, $139,000)
4. SOF/LDV 12–24 weeks depending on cirrhosis (96%/96%, $87,000–175,000)

In sensitivity analyses we considered only treating patients with SOF/LDV when they developed metavir F2 fibrosis.
Results: *Treatment naïve patients with and without cirrhosis:* SOF/LDV increased QALE (0.5–9.1 QALY) and cost ($29,676-$68,118), resulting in ICERs $11,800–63,900/QALY (Table). Short SOF/LDV treatment (8 weeks) in non-cirrhotic patients with HCV RNA <6 million resulted in SOF/LDV being cost saving compared to other regimens.
Treatment-experienced patients without cirrhosis: SOF/LDV was cost saving compared to other regimens.
Treatment-experienced patients with cirrhosis: Compared to SOF/SMV 12 weeks, SOF/LDV 24 weeks increased cost by $20,975 and had an ICER <$100,000/QALY.
Sensitivity analyses: Including the SOF/LDV F2 strategy did not change cost-effectiveness conclusions. Waiting for F2 disease resulted in lower QALE than treating all patients due to quality of life decrements while waiting for treatment and was a dominated strategy.
Conclusions: SOF/LDV improves outcomes and has an ICER <$100,000/QALY for treatment naïve and experienced patients with and without cirrhosis. Limiting SOF/LDV access to only F2 disease results in lower QALE than simply treating all patients, at either higher total cost, or a higher cost per QALY gained.

P0799

THE PHARMACOKINETICS OF BECLABUVIR (BMS-791325) WHEN ADMINISTERED IN COMBINATION WITH DACLATASVIR AND ASUNAPREVIR IN TREATMENT-NAIVE PATIENTS WITH OR WITHOUT CIRRHOSIS INFECTED WITH HCV GENOTYPE 1

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Background and Aims: Daclatasvir 30 mg (DCV; NS5A inhibitor), asunaprevir 200 mg (ASV; NS3 inhibitor), and beclabuvir 75 mg (BCV, BMS-791325; non-nucleoside NS5B inhibitor), administered twice daily (BID) as a fixed-dose combination (DCV/ASV/BCV) is currently in phase 3 development for the treatment of hepatitis C virus (HCV) infection. This DCV/ASV/BCV regimen administered for 12 weeks achieved a 100% sustained virologic response rate in HCV genotype (GT) 1-infected treatment-naive patients with compensated cirrhosis (Child–Pugh A) in an open-label, multiple-dose phase 2 study (AI443014); pharmacokinetics (PK) of DCV, ASV, BCV, and BMS-794712, an active metabolite of BCV in patients with and without cirrhosis are presented here.
Methods: Patients (age, 18–70 years; BMI, 18–35 kg/m^2) infected with HCV GT1 with (n = 8) or without (n = 73) biopsy-confirmed compensated cirrhosis (Child–Pugh A; capped at 10% of treatment group) received DCV/ASV/BCV for 12 weeks. Blood samples (0–12 h) were collected post-AM dose on days 1 and 14 from the first 20 non-cirrhotic patients and all cirrhotic patients for analysis by LC-MS/MS; standard non-compartmental PK parameters were calculated and summarized by descriptive statistics. Comparisons of (1) DCV and ASV PK descriptive statistics with PK data from Study AI447011 (DCV + ASV administered in combination in HCV-infected patients without cirrhosis), and (2) BCV and BMS-794712 PK descriptive statistics with PK data from Study AI443012 (BCV + pegIFNα/RBV in HCV-infected patients) will also be presented. In addition, data from patients receiving a DCV/ASV/BCV regimen containing BCV 150 mg BID will be presented.
Results: BCV and BMS-794712 steady state exposures were similar between patients with and without compensated cirrhosis (Table). The results of DCV and ASV steady state exposures in cirrhotic versus non-cirrhotic patients were consistent with historical

observations. DCV/ASV/BCV treatment was generally well tolerated in patients with and without compensated cirrhosis.
Conclusions: Data indicate that the presence of compensated cirrhosis (Child–Pugh A) does not have any clinically meaningful effect on exposures of BCV and BMS-794712, and similar safety profiles were observed in patients with or without cirrhosis.

Analyte	Study AI443014 AUC_{TAU}, ng·h/mL (Day 14)	
	Non-cirrhotic, n = 18	Cirrhotic, n = 8
BCV	9711 (44)	8304 (38)
BMS-794712	2145 (42)	2783 (50)

Data are geometric mean (CV%). AUC_{TAU}, area under the plasma concentration time curve during the dosing interval (0–12 h).

P0800
EFFECTIVENESS AND SAFETY OF COMBINATION NEW REGIMENS BASED ON SOFOSBUVIR (SOFO) WITH RIBAVIRIN (RBV), DACLATASVIR (DAC) OR SIMEPREVIR (SIM) IN DIFFICULT PATIENTS TO TREAT INFECTED WITH CHRONIC HEPATITIS C (CHC)

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Background and Aims: SOFO, DAC and SIM have been approved for treatment of CHC patients with advanced fibrosis in combination with or without Ribavirin (RBV). Beside study results, limited data are available for these drugs during treatment under real life conditions. Further data are necessary to reflect the effectivity and safety of IFN-free therapy in difficult to treat patients (pts).
Methods: This prospective study conducted by the referral hospital Beaujon, France including 317 CHC pts between Jan to 20 November 2014, 29 pts excluded for missing data and 288 treated with SOFO+RBV (n = 136), SOFO+DAC (n = 104) or SOFO+SIM (n = 48). This cross sectional analysis included HCV monoinfected with G1/4 with fibrosis F3–F4 in each combination respectively (n = 92/ 60), (n = 72/32), (n = 29 and 19). At point of time of analysis, 288 pts reached week 12, 55 completed treatment, and 138 pts at follow up week +4.
Results: Baseline characteristics in total were age of 51 yrs, 72% male, BMI 25 kg/m^2, median HCV-RNA (5.4 log), GT-1 (70%), Fibrosis stage F3–F4 (64%). Responses to treatments and side effects are reported in the table.
Conclusions: Despite of the severity of liver disease combinations of IFN free regimens based Sofosbuvir seems to be effective with a good tolerability in this real-life. Patients accede to new IFN free regimens with less adverse events compared to IFN regimen. Effective management of new treatment combinations is necessary to reach optimal results in clinical practice.

	SOFO + RBV (n = 136)	SOFO + DAC (n = 104)	SOFO + SIM (n = 48)
Response to different antiviral regimens			
SVR at Week 12	134/136 (98%)	104/104 (100%)	45/48 (94%)
SVR at Week +4 of follow up	75/80 (94%)	41/42 (98%)	14/16 (87%)
Main side effects reported during treatment			
Photosensitivity reaction			6 (12%)
Asthenia	11 (8%)	6 (6%)	
memory problems	3 (2%)	5 (5%)	
Irritability	5 (4%)	4 (4%)	2 (4%)

P0801
ERADICATE HCV: TREATMENT AS PREVENTION IN CHAOTIC DRUG USERS

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Background and Aims: Approximately 1% of the Scottish population have been infected with hepatitis C compared to around 0.5% in other parts of the UK. Prevalence of Hepatitis C virus (HCV) in people who inject drugs (PWID) in Tayside has been found to be consistently around 30%. Modelling work of Martin et al (2011) raises the possibility that prevalence of HCV may be reduced by treating relatively small numbers of active drug users and therefore preventing onward transmission. The model illustrates that treating as few as 10 per 1000 drug users per year can significantly impact on prevalence rates.
This group has been previously thought to be too chaotic to adhere to therapy. The first step to "Treatment as Prevention" is to treat these patients.
Objectives: To recruit 100 hepatitis C positive PWID, offer HCV treatment, record sustained virological response (SVR) rates obtained and monitor any long term population prevalence changes.
Methods: To engage PWID at the needle exchange centres in Tayside. Incentivise suitable participants to comply with treatment of Interferon/Ribavirin and a protease inhibitor, if required. Giving them £5–10 grocery vouchers and high protein drinks to attend on a weekly basis throughout the course of their treatment and follow up.
Results: In the initial 20 month study period, 119 patients discussed the study with the specialist nurses. Of those, 46 were not eligible for treatment. Reasons for ineligibility; 20 were PCR negative, 15 had no contraceptive, 3 were HCV anti-body negative, 2 had no genotype, 2 were drug free, 2 were previously treated, 1 became pregnant and 1 had unstable mental health. Of the 73 eligible patients, 51 agreed to participate and were consented. Of the 51 consented, 38 (74.5%) were male. 41 have since commenced treatment, 3 being lost to follow up, 2 are in prison, 1 has a start date pending, 2 are now drug free and are on treatment via the standard pathway, 1 in hospital and 1 had no contraception.

Table 1. Basic demographics and results

	Total (n)
Total number	51
Genotype 1	17 (33.3%)
Genotype 3	34 (66.7%)
Viral load <600,000 iu/ml	37/51 (72.5%)
Treatment started	41
PCR(week 4)	41/41 ≤16 iu/ml
Treatment stopped	3 (2 obtained SVR)
Treatment completed	22
Non-responder/relapser	2
3 month SVR	17/20 (85%)
6 month SVR	12/15 (80%)

Conclusions: The results to date show that it is possible to connect with this chaotic PWID population. All of this population reached HCV PCR ≤16 iu/ml by week 4, culminating in an SVR rate of 80% at 6 months post treatment. The incentivisation scheme is effective in the initial engagement stage, however once on treatment the paramount importance of becoming cured becomes the primary focus.

P0802

SAFETY AND EFFICACY OF SOFOSBUVIR + SIMEPREVIR WITHOUT RIBAVIRIN IN HEPATITIS C GENOTYPE 1-INFECTED PATIENTS WITH END-STAGE RENAL DISEASE OR GFR <30 mL/min

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Background and Aims: Treatment of chronic hepatitis C (CHC) in the setting of ESRD and/or severe renal impairment has historically been limited by poor tolerability and low cure rates regardless of the regimen used. Combination antiviral therapy using sofosbuvir & simeprevir is now an approved treatment option in patients with genotype 1 CHC; however, the safety and efficacy of this regimen in patients with ESRD on HD or with GFR <30 mL/min is not known. Interim data from a combined treatment cohort of two large hepatology referral centers were evaluated to assess for safety and efficacy of full dose sofosbuvir + simeprevir without ribavirin in this special patient population.
Methods: All (n = 12) patients included in the analysis had severe renal impairment including ESRD on HD (n = 11) or GFR <30 mL/min (n = 1). All received sofosbuvir 400 mg daily + simeprevir 150 mg daily without ribavirin, for anticipated course of 12 weeks. Interim safety and efficacy data are presented. SVR4 and SVR12 data for all patients to follow.
Results: Of the 12 patients in this cohort, 11 (92%) were male, 7 (58%) were cirrhotic, 3 (25%) had stage 3 liver fibrosis, 10 (83%) were genotype 1A, and 10 (83%) were treatment naïve. Eight patients (67%) have completed 12 weeks of therapy. The remaining 4 patients have been treated for a median of 9 weeks (range 3–11). The mean baseline hemoglobin was 11.6 g/dL and 6 patients were on Epoetin since prior to starting therapy. Treatment was overall well tolerated with no treatment discontinuations reported. Five (41%) patients reported adverse events (AE), 4 of which were rated as mild clinical manifestations, and one a laboratory abnormality. These AEs were insomnia (n = 2), headache (n = 1), nausea (n = 1), and worsening anemia requiring blood transfusion (n = 1). All 8 (100%) patients who completed 12 weeks of therapy achieved end of treatment response (negative HCV RNA at week 12). Of the 7 patients that have reached post-treatment week-4 follow up, 100% were HCV RNA negative (SVR4). All 5 (100%) patients who reached post-treatment week-12 follow up, achieved SVR12 or virologic cure.
Conclusions: Daily, full dose of sofosbuvir plus simeprevir for 12 weeks of therapy appears to be well tolerated in patients with ESRD on HD or GFR <30 mL/min. Most common AEs resembled those of healthier CHC patients without significant renal impairment. 100% of patients who completed therapy had EOT.

P0803

COST-EFFECTIVENESS OF LEDIPASVIR/SOFOSBUVIR FOR THE TREATMENT OF GENOTYPE 1 OR 4 CHRONIC HEPATITIS C IN SCOTLAND

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Background and Aims: Ledipasvir/sofosbuvir (LDV/SOF) is a new all-oral fixed-dose combination tablet indicated for the treatment of chronic hepatitis C (CHC) in adults. LDV/SOF has demonstrated overall SVR rates of >94% and discontinuation rates <1% in the phase 3 clinical trial programme. As part of the Health Technology Appraisal (HTA) process in Scotland an economic model was developed to estimate the cost-effectiveness of LDV/SOF for genotype (GT) 1 or 4 compared to standard of care regimens.
Methods: A decision-analytic Markov model was used to evaluate the cost-effectiveness of LDV/SOF for 8, 12 or 24 weeks compared to sofosbuvir (SOF) or simeprevir (SMV) in combination with pegylated interferon (PEG-IFN) and ribavirin (RBV) for 12 or 24–48 weeks duration, respectively. In addition, a comparison was made versus SMV+SOF for patients who are ineligible for interferon. The model was constructed based on precedent models for the treatment of CHC, and evaluated treatment naïve and treatment experienced patients separately. Patients entered the model in either a non-cirrhotic or cirrhotic disease state. Patients who were successfully treated, and therefore achieved an SVR, moved to the corresponding SVR state. Patients who did not achieve an SVR were exposed to a risk of CHC disease progression, liver transplant or liver-related mortality. Cost and utility data were included in the model associated with the treatment phase and disease state. The model outcomes included the incremental cost-effectiveness ratio (ICER) reported in terms of cost per incremental quality-adjusted life year (QALY) in accordance with Scottish guidelines.
Results: In CHC GT1 treatment naïve patients, LDV/SOF dominated SOF+PEG-IFN+RBV and SMV+PEG-IFN+RBV, achieving both cost-savings and higher QALYs. In GT4 treatment naive patients, LDV/SOF was cost-effective compared to SOF+PEG-IFN+IFN and SMV+PEG-IFN+RBV with ICERs of £4,088 and £12,651 respectively. In treatment experienced GT1 and GT4 patients, LDV/SOF had ICERs of £5,894 compared to SOF+PEG-IFN+RBV and £9,788 compared to SMV+PEG-IFN+RBV. LDV/SOF dominated SMV+SOF in all scenarios.
Conclusions: LDV/SOF is a highly effective and cost-effective single tablet regimen for patients with CHC GT1 or GT4 in Scotland compared to SOF+PEG-IFN+RBV and SMV-based regimens. The first generation protease inhibitors were not included as they have similar efficacy and cost compared to SMV, therefore LDV/SOF is also cost-effective compared to these medicines.

P0804

EFFICACY OF SOFOSBUVIR-BASED TREATMENT REGIMENS IN HIV/HCV CO-INFECTED PATIENTS AFTER LIVER TRANSPLANTATION: THE ANRS CO23 CUPILT STUDY

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Background and Aims: Severe hepatitis C virus (HCV) recurrence after liver transplantation (LT) affects post-transplant survival in HIV/HCV co-infected patients. The recent approval of interferon-free regimen using direct antiviral agents (DAA) has radically changed the management of recipients. Study aims were to assess efficacy and safety of based sofosbuvir (SOF)-based treatment regimens in HIV/HCV coinfected patients after LT.
Methods: The CUPILT study is a prospective nationwide cohort including patients with HCV-recurrence following LT treated by new antivirals. The present work focused on 16 HIV/HCV coinfected patients and included between August 2013 and August 2014 in 10 centers. Treatment regimens were prescribed at investigator's discretion.
Results: This cohort study enrolled 16 liver transplant patients (male: 81%, mean age 53±5 years [46–62]). Seven patients (43%)

were non-responders to a previous course of therapy post-LT (Peg-IFN/RBV n = 5; 1st generation PI based regimen n = 2). At W0 the proportion of G1, G3, G4, and median levels of gGT, haemoglobin, HCV viral load (VL), HIV VL and CD4 count were: 56%, 6%, 19%, 476.2±328.2 IU/L [49–939], 12.8±2 g/dL [9.2–15.3], 7.0±0.6 $\log_{10}$ IU/mL [6.2–8.4], <40 cp/ml and 301±177 cells/mm^3 [122–671], respectively. Indication for treatment was HCV recurrence (≥F1, n = 12) or fibrosing cholestatic hepatitis (n = 4). Anti HCV-therapy started at 29.2±33.8 months [3.2–126.3] post LT. The regimens used were as follows: Peg-IFNa + SOF + RBV (n = 1), SOF + RBV (n = 3), SOF + DCV (n = 3) and SOF + DCV + RBV (n = 9) for 24 weeks (n = 13). RBV was combined to SOF + DCV in 13 patients. At W4, HCV VL was <15 IU/mL in 3 patients (19%). To date, 10 patients (53%) have stopped treatment and all of them have an undetectable HCV VL (<15 IU/mL). Seven patients (44%) acquired SVR12. Five patients (31%) developed serious adverse events (haematological n = 7, sepsis n = 2 and renal insufficiency n = 1). No significant drug–drug interaction was observed. Complete results of safety and efficacy with SVR 24 for all patients will be presented.

Conclusions: Anti HCV treatment with sofosbuvir and daclatasvir show excellent results in HIV/HCV coinfected patients after liver transplantation.

P0805

ACHIEVEMENT OF SVR12 DESPITE THE PRESENCE OF HCV VARIANTS RESISTANT TO FIRST GENERATION NS5A INHIBITORS IN GENOTYPE-1 HEPATITIS C PATIENTS AFTER 8-WEEK THERAPY OF ACH-3102 IN COMBINATION WITH SOFOSBUVIR

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Background and Aims: ACH-3102 is a second-generation HCV NS5A inhibitor that retains activity against multiple variants resistant to first-generation NS5A inhibitors such as ledipasvir. In a phase 2a study, sustained virologic response 12 weeks after therapy (SVR12) was achieved by 100% of genotype-1 hepatitis C patients after 8-week administration of ACH-3102 and sofosbuvir. Here we report genotypic and phenotypic analyses of baseline samples collected from patients in this study.

Methods: NS5A fragments were PCR-amplified from patient plasma samples collected at baseline and sequenced either directly or after cloning. Chimeric replicons incorporating NS5A fragments from patient plasma samples were used for phenotypic analyses.

Results: Mutations at positions associated with viral resistance to first-generation NS5A inhibitors were detected in baseline samples. In particular, mutations at positions associated with virologic failures of ledipasvir and sofosbuvir combination treatment were detected as major species by population sequencing in 50% of both genotype-1a (5 out of 10) and genotype-1b (1 out 2) baseline samples. These positions include NS5A amino acid residues 24, 28, 30, 31 and 58. Phenotypic analysis confirmed that genotype-1a and 1b mutations at these positions as well as genotype-1b mutations at NS5A amino acid residue 93 retain significantly greater susceptibility to ACH-3102 than to ledipasvir. This greater susceptibility may have contributed to the success of the ACH-3102-containing regimen, although additional confirmatory studies are needed, especially in types of hepatitis C patients whose viral baseline NS5A sequences compromise virologic responses to ledipasvir and sofosbuvir combination treatment.

Conclusions: Despite the presence of viral variants resistant to ledipasvir as major species at baseline, 100% SVR12 was achieved in genotype-1 hepatitis C patients after 8-week administration of ACH-3102 and sofosbuvir. These results suggest that ACH-3102 in combination with an NS5B nucleotide inhibitor may improve clinical efficacy against HCV variants with resistance to first-generation NS5A inhibitors.

P0806

THE VALUE OF SURVIVAL BENEFITS FROM TREATING HEPATITIS C AT DIFFERENT FIBROSIS STAGES WITH ALL-ORAL, INTERFERON-FREE THERAPY RELATIVE TO 'WATCHFUL WAITING'

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Background and Aims: Chronic infection with the hepatitis C virus (HCV) may lead to complications that negatively affect the length and quality of life. The AbbVie 3D regimen is an all-oral, interferon-free therapy for treating patients with chronic genotype 1 (GT1) HCV that has demonstrated high effectiveness in achieving a sustained virologic response (SVR) in Phase III clinical trials. However, the survival benefits of treatment with this regimen

Table (abstract P0806): Survival benefits of AbbVie 3D regimen versus no treatment among patients with chronic GT1 HCV

	Discounted LYs				Discounted QALYs			
	AbbVie 3D	No treatment	Difference (AbbVie 3D – No treatment)	Survival benefit evaluated at $100k/LY	AbbVie 3D	No treatment	Difference (AbbVie 3D – No treatment)	Survival benefit evaluated at $100k/QALY
Treatment naive								
F0	18.20	17.57	0.63	$62,987	17.98	16.18	1.80	$180,460
F1	18.12	16.98	1.15	$114,746	17.82	14.96	2.86	$285,545
F2	18.01	16.07	1.94	$193,751	16.54	13.28	3.26	$326,454
F3	17.82	14.50	3.31	$331,237	16.35	11.78	4.57	$456,748
F4	15.59	11.60	3.99	$398,766	14.18	9.10	5.09	$508,723
Overall*	17.82	16.16	1.66	$165,673	17.11	14.06	3.05	$304,758
Treatment experienced								
F0	17.43	16.81	0.61	$61,432	17.23	15.44	1.78	$178,367
F1	17.36	16.25	1.11	$111,220	17.08	14.29	2.79	$278,951
F2	17.26	15.40	1.86	$186,350	15.86	12.71	3.16	$315,912
F3	17.10	13.97	3.12	$312,360	15.70	11.34	4.36	$436,084
F4	15.11	11.34	3.77	$376,524	13.77	8.89	4.88	$487,885
Overall**	16.67	14.58	2.09	$209,343	15.84	12.43	3.41	$340,716

*Based on a distribution of 27% F0, 36% F1, 17% F2, 9% F3 and 11% F4 among treatment-naive patients with HCV.
**Based on a distribution of 20% F0, 27% F1, 13% F2, 10% F3 and 30% F4 among treatment-experienced patients with HCV.

relative to 'watchful waiting' (i.e. no HCV treatment) at different fibrosis stages are not well understood. The aim of this study is to quantify these survival benefits in terms of life-years (LYs) and quality-adjusted life years (QALYs) and to monetize their value.

Methods: A Markov disease utility state-transition model was developed to estimate survival benefits accruing from no HCV treatment and treatment with the AbbVie regimen from the perspective of a private US payer. Survival outcomes were evaluated over a lifetime horizon with annual cycles and discounted at 3% per year. Transition probabilities and health utilities associated with 14 health states were obtained from published literature. The model population was composed of treatment-naïve patients aged 52 years and treatment experienced patients aged 54 years with GT1 chronic hepatitis C. About 60% of the patient population was male. Clinical efficacy and disutilities associated with HCV treatment were extracted from AbbVie trials. Survival benefits were monetized based on an assumed valuation of $100,000 per LY or QALY suggested in the literature.

Results: Compared to watchful waiting, the lifetime survival benefits of HCV treatment with the AbbVie regimen vary substantially by stage of treatment initiation. For treatment-naive patients, the value of survival benefits increases six-fold from $62,987 with initiation at F0 to $398,766 with initiation at F4 (see Table). The quality-adjusted value of these survival benefits increases nearly three-fold from $180,460 with initiation at F0 to $508,723 with initiation at F4. Regardless of the fibrosis stage when treatment is initiated, treatment-experienced patients have 1%>6% less survival benefits than treatment-naïve patients.

Conclusions: The AbbVie 3D regimen prolongs the survival of chronic HCV patients and improves quality of life. The survival costs of watchful waiting are substantially greater at more advanced levels of liver fibrosis.

P0807

THE QUALITY OF LIFE JOURNEY FOR PATIENTS WITH CHRONIC HEPATITIS C: FROM INTERFERON AND RIBAVIRIN TO INTERFERON-FREE AND RIBAVIRIN-FREE REGIMENS

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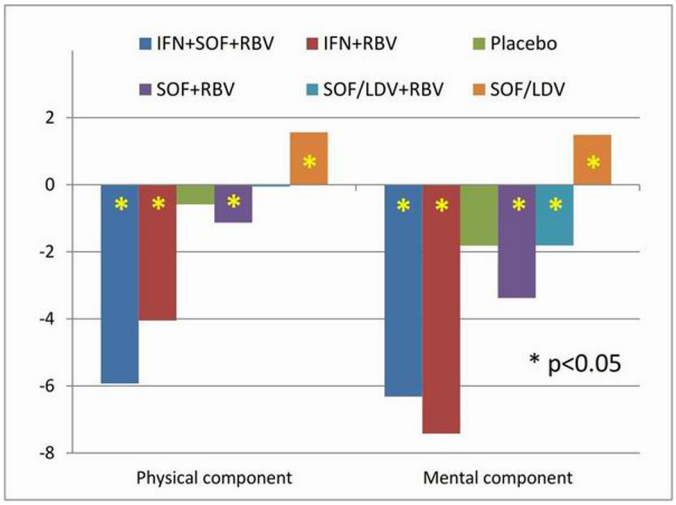
Background and Aims: Interferon (IFN) and ribavirin (RBV) negatively impact health-related quality of life (HRQL) during treatment. The aim is to compare the impact of IFN- and/or RBV-containing regimens on HRQL to that of IFN- and RBV-free regimens.

Methods: HRQL data from nine multicenter multinational phase 3 clinical trials of sofosbuvir (SOF)-based regimens with and without ledipasvir (LDV), IFN, or RBV (POSITRON, FISSION, FUSION, NEUTRINO, VALENCE, PHOTON-1, ION-1, ION-2, and ION-3) were used. The Short Form-36 (SF-36) HRQL questionnaire was administered to subjects at baseline, during and 12 weeks after treatment.

Results: A total of 3,506 CH-C patients were included (age: 52.2±10.2 years old, 62.2% male, 75.8% enrolled in the U.S., 73.7% treatment-naïve, 15.1% cirrhotic). Of these, 327 received IFN+RBV+SOF, 110 received IFN+RBV, 1,046 received SOF+RBV, 872 received SOF/LDV+RBV, 1,080 received SOF/LDV and 71 received placebo. At the last day of treatment, severe decrements in HRQL were noted in IFN+RBV±SOF [Physical component score (PCS): −11.6 to −16.9%, $p < 0.0001$; mental component score (MCS): −13.2% to −15.6%, $p < 0.0001$], while moderate decrements were noted in SOF+RBV±LDV [PCS: −3.2% $p < 0.0001$, MCS: −3.8 to −7.1%, $p < 0.0001$] (Figure 1). In contrast, in SOF/LDV regimen, improvements in PCS (+4.8%) and MCS (+4.3%) were noted ($p < 0.0001$). By SVR12, HRQL returned to baseline in IFN+RBV±SOF ($p > 0.05$) and improved in all IFN-free arms: PCS +3.6% to +4.8%, and MCS +2.9% to +4.5% (all $p < 0.0001$). Post-SVR12 improvements were noted in all individual scales of SF-36 in SOF/LDV, ranging between +2.9% (role emotional) and +7.8% (vitality) (all $p < 0.0001$). In multivariate analysis, end of treatment PCS score was lower in IFN+RBV±SOF (beta −10.4 to −15.9%, $p < 0.0001$), and higher in SOF/LDV (beta +6.0%, $p < 0.0001$) compared to the reference (SOF+RBV). Similarly, end of treatment MCS score was lower in IFN+RBV-containing regimens (beta −6.4% to −9.2%), slightly higher in SOF/LDV+RBV (beta +3.3%), and substantially higher in SOF/LDV (beta +10.2%) (all $p < 0.001$). Finally, by SVR12, SOF/LDV was associated with higher MCS (+2.5%, $p < 0.001$).

Conclusions: Removing IFN and RBV has led to substantial improvement of HRQL during treatment. Treatment with IFN- and RBV-free anti-HCV treatment (LDV/SOF) is independently associated with better HRQL during and after treatment.



P0808

IMPROVEMENT IN LIVER FUNCTION AND NON-INVASIVE ESTIMATES OF LIVER FIBROSIS 48 WEEKS AFTER TREATMENT WITH OMBITASVIR/PARITAPREVIR/R, DASABUVIR AND RIBAVIRIN IN HCV GENOTYPE 1 PATIENTS WITH CIRRHOSIS

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Background and Aims: Viral eradication in individuals with chronic HCV infection and cirrhosis may result in improvement in hepatic synthetic function and reduce portal hypertension. The interferon-free 3 direct-acting antiviral (3D) regimen of ombitasvir, paritaprevir (identified by AbbVie and Enanta, dosed with ritonavir [r]), and dasabuvir with ribavirin (RBV) has shown high rates of sustained virologic response rates 12 weeks post-treatment (SVR12) in HCV genotype 1-infected patients with cirrhosis in the TURQUOISE-II trial. We evaluated various non-invasive markers of liver function and fibrosis 48 weeks after treatment with 3D + RBV.

Methods: In this open-label trial, patients were randomized to receive 3D + RBV for 12 or 24 weeks. Changes in non-invasive estimates of liver fibrosis [AST to platelet ratio (APRI), FIB-4, Forns Index, FibroTest], laboratory surrogates for hepatic synthetic function [international normalized ratio (INR), albumin, platelet count], and alpha fetoprotein (AFP) levels were measured over time.

Results: Among 380 patients randomized to 12 or 24 weeks of 3D + RBV, SVR12 rates were 92% and 97%, respectively. In general, mean non-invasive estimates of liver fibrosis, surrogates for hepatic synthetic function, and AFP improved from baseline to post-treatment week 12 (PTW12), and further improved by post-treatment week 48 (PTW48; Table). In patients treated for 12 and 24 weeks who achieved an SVR12, mean FibroTest scores improved to 0.64 and 0.65 at PTW48, respectively. Conversely, in patients who did not achieve SVR12 after 12 and 24 week treatment, mean FibroTest scores increased to 0.89 and 0.89, respectively. Results by baseline disease severity will be presented.

Conclusions: In the phase 3 TURQUOISE-II trial of patients with HCV genotype 1 and cirrhosis, treatment with the 3D + RBV regimen improved surrogates of hepatic synthetic function, AFP levels, and FibroTest scores after completion of antiviral therapy in patients with HCV genotype 1 infection and cirrhosis.

	3D + RBV for 12 weeks				3D + RBV for 24 weeks			
	N [a]	BL Mean	PTW12 Mean	PTW48 Mean	N [a]	BL Mean	PTW12 Mean	PTW48 Mean
Albumin (g/dL)	193	3.92	4.12	4.22	163	3.93	4.19	4.37
Platelet count ($\times 10^9$/L)	188	149.9	155.2	162.9	163	148.9	159.3	160.2
Total bilirubin (mg/dL)	193	0.85	0.71	0.74	163	0.87	0.65	0.68
FibroTest score	161	0.81	0.69	0.66	149	0.81	0.67	0.66
Pts w/ SVR12	152	0.80	0.68	0.64	146	0.81	0.67	0.65
Pts w/o SVR12	9	0.91	0.82	0.89	3	0.79	0.84	0.89
INR	189	1.08	1.08	1.07	161	1.04	1.07	1.06
AFP (ng/mL)	116	15.69	5.70	5.49	163	21.57	4.89	4.72
APRI	185	2.01	0.74	0.67	160	2.10	0.61	0.57
FIB-4	185	4.20	2.65	2.50	160	4.14	2.31	2.18
Forns Index	186	7.73	6.53	6.29	160	7.70	6.28	6.24

AFP, alpha fetoprotein; APRI, AST to Platelet Ratio Index; BL, baseline; INR, international normalized ratio; Pts, patients; PTW, post-treatment week.
[a] N's are based on the number of patients with PTW48 data; BL means are for N patients; PTW12 means are for patients with data available at that time point, not necessarily N.

P0809

98% SVR12 IN KOREAN AND TAIWANESE PATIENTS WITH CHRONIC GENOTYPE 2 HCV INFECTION RECEIVING 12 WEEKS OF SOFOSBUVIR PLUS RIBAVIRIN: RESULTS FROM AN INTERNATIONAL, MULTICENTER PHASE 3 STUDY

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Background and Aims: Infection with genotype (GT) 2 hepatitis C virus (HCV) accounts for approximately half of all cases of chronic HCV infection in Korea and Taiwan. Patients have often failed to respond to prior interferon (IFN)-based therapy and may be ineligible for, or intolerant of current available therapy. There is a significant unmet medical need for highly effective, safe and well-tolerated IFN-free therapies for use in this aging population and those with advanced liver disease.

Methods: An open-label, Phase 3 study was conducted to evaluate the efficacy and safety of sofosbuvir (SOF) 400 mg once daily plus weight-based ribavirin (RBV; 1000–1200 mg/day) administered for 12 weeks in treatment-naïve and treatment-experienced Korean and Taiwanese adults with chronic GT2 HCV infection, with and without cirrhosis. No upper age limit was applied and in order to support enrollment of patients with cirrhosis no entry restriction applied for neutrophils and the minimum platelet count was 50,000/μL. NS5B resistance associated variants were evaluated by deep sequencing. The primary efficacy endpoint was SVR12.

Results: 216 patients were enrolled. The mean (range) age was 54 (22–82) years old, BMI 24 (18–38) kg/m^2, and baseline HCV RNA 6.2 (2.1–7.7) $\log_{10}$ IU/mL. The majority of patients were treatment-naïve (69%), non-cirrhotic (88%), female (57%), and had IL28B CC genotype (84%). Table 1 shows the overall SVR12 rates. A total of 4 patients (2%) failed to achieve SVR12: 1 patient had on-treatment breakthrough, 1 patient relapsed and 2 patients' were lost to follow-up. No S282T or SOF treatment emergent variants (L159F or V321A) were detected in the 2 virologic failure patients at baseline or at the time of virologic failure. Adverse events (AE) reported in ≥10% of patients were pruritus, headache, and insomnia. Six patients (3%) had treatment-emergent SAEs all unrelated to study drug. No patients discontinued therapy due to an AE. Overall, 11% of patients had hemoglobin <10 g/dL and 1% had hemoglobin <8.5 g/dL during treatment. No other laboratory abnormalities were observed. Baseline viral sequencing results will also be presented.

Table 1. SVR12 rates in GT2-infected patients and difficult-to-treat subgroups

	Korea (N = 129)	Taiwan (N = 87)	Overall (N = 216)
Virologic response (ITT)			
Overall, SVR12, n (%)	125 (97)	87 (100)	212 (98)
On-treatment failure, n (%)	1 (1)	0	1 (<1)
Relapse, n (%)	1 (1)	0	1 (<1)
Lost to follow-up, n (%)	2 (2)	0	2 (1)
SVR12 by subgroup			
Treatment-experienced (TE), n/N (%)	24/24 (100)	44/44 (100)	68/68 (100)
Cirrhotic, n/N (%)	13/13 (100)	13/13 (100)	26/26 (100)
TE with cirrhosis, n/N (%)	7/7 (100)	9/9 (100)	16/16 (100)

Conclusions: Sofosbuvir plus RBV for 12 weeks is highly effective and well tolerated in Korean and Taiwanese patients with GT2 HCV infection, including those with cirrhosis.

P0810

SOFOSBUVIR AND DACLATASVIR COMBINED TREATMENT FOR CHRONIC HEPATITIS C: FIRST-YEAR EFFICACY AND SAFETY DATA IN A REAL-LIFE SETTING

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Background and Aims: The combination of sofosbuvir and daclatasvir (SoDa) has not largely been evaluated in real-life settings. The aim of this study was to evaluate the efficacy and safety in patients with chronic hepatitis C treated with SoDa in general hospitals in France.

Methods: APROVVIE 2 is a longitudinal observational study of patients treated with new direct antiviral agents in French general hospitals (first in early access programs, then following market authorizations). Demographic, clinical, adverse events, biological and virological data are prospectively collected throughout treatment and post treatment follow-up from willing patients by their responsible physicians, voluntary investigators, members of the Association Nationale des Gastroentérologues des Hôpitaux généraux (ANGH).

Results: Since March, 2014, 140 patients with chronic hepatitis C starting treatment with SoDa were included by 13 investigators in 12 centers. Main characteristics of the patients were: male gender 58%, median age 57 years, cirrhosis (C) 54% (Child A 85%, Child B 9%), F3 fibrosis stage 32%, high viremia 68%, patients naïve 22%, treatment-experienced 78% [non-responders 43% (including null responders 15%), relapsers 35%], genotype (G) 1: 74%, G 3: 15%, G 4: 7%, G 5: 4%.

Daily doses were 400 mg/j of sofosbuvir and 60 mg of daclatasvir, except in the 7 HIV-coinfected patients in whom daclatavir dosage was modified according to the antiretroviral combination used; ribavirin was prescribed in only 2 patients.

Available on-treatment responses, similar in patients with or without cirrhosis, are described below.

G 1 + 5 (n = 109): C 56% (Child A/B: 91%/9%), Patients (Pts) naïve 21%, Viral response (undetectable HCV-RNA) week 2 (VR2) 33%, Viral Response week 4 (VR4) 75%, Viral response week 12 (VR12) 89%.

G 3 (n = 21): C 50% (Child A/B: 86%/4%), Pts naïve 29%, VR2 55%, VR4 64%, VR12 100%.

G 4 (n = 10): C 40% (Child A 100%), Pts naïve 20%, VR2 50%, VR4 75%, VR12 100%.

To date, no grade 3–4 adverse effect has been reported.

Conclusions: In a population of patients mainly treatment-experienced and with cirrhosis or severe fibrosis, treated with sofosbuvir and daclatasvir initial tolerance and efficacy data seem to be in the range of phase II studies. Complete SVR and safety data will be presented.

P0811

SOFOSBUVIR-BASED DIRECT-ACTING ANTIVIRALS TREATMENT FOR ELDERLY CHRONIC HEPATITIS C PATIENTS: A COST-EFFECTIVENESS ANALYSIS

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Background and Aims: A relevant proportion of patients affected by Chronic Hepatitis C (CHC) is older than 65 years. In the interferon era, comorbidities and a higher susceptibility to interferon/ribavirin adverse events have historically limited treatment in these patients. Recent approval of interferon-free regimens, characterized by high efficacy and limited toxicity, provide unprecedented chances for these patients to receive curative treatments. However, the cost-effectiveness of all-oral Direct-Acting Antivirals (DAAs) has not been addressed in the elderly population. We have performed a cost-effectiveness analysis taking into account the severity of liver disease, the age of the patient and the geriatric (frailty) status.

Methods: A semi-Markov model of CHC natural history was built. The study focuses on CHC patients older than 65 years, stratified according to liver fibrosis (METAVIR F3 and F4), age (65 to 85 years old) and frailty phenotype defined by Fried's (not frail, pre-frail and frail) for a total of 30 cohorts simulated. Treatment with sofosbuvir plus simeprevir (SOF/SMV) combination versus no treatment was assessed for each cohort population. The model estimated costs, Life Years and Quality Adjusted Life Years (QALY) using a lifetime time horizon and the Health System perspective. Results are presented as incremental cost-effectiveness ratios (ICERs) per QALY gained. Cost-effectiveness was defined as an ICER under the willingness-to-pay threshold of €37,000 per QALY gained.

Results: At each fibrosis score, ICER increased with age and frailty index. Among patients with F3 fibrosis, ICER ranged from €13,934/QALY in not-frail 65 years old and €79,354 in frail 85 years old patients. Among F4 patients ICER ranged from €13,873/QALY in not frail 65 years old and €115,965 in frail 85 years old patients. In both F3 and F4 cohorts ICER was below €37,000/QALY up to age 80 in non-frail pts, up to age 75 in pre-frail patients, up to age 70 in frail patients. Adopting an alternative scenario with a 20% discount of SOF/SMV treatment, the number of cohort population simulated with an ICER below € 37,000/QALY increased.

Conclusions: SOF/SMV treatment is cost-effective in most CHC patients older than 65 years, however a careful assessment of the patient geriatric status is mandatory. This cost-effectiveness analysis should promote a prospective clinical study to verify efficacy and side effects in elderly HCV patients.

P0812

IMPACT OF SVRW12 IN THE DEVELOPMENT OF COMPLICATIONS AND FIBROSIS REGRESSION IN PATIENTS TREATED WITH TRIPLE THERAPY WITH BOCEPREVIR AND TELAPREVIR

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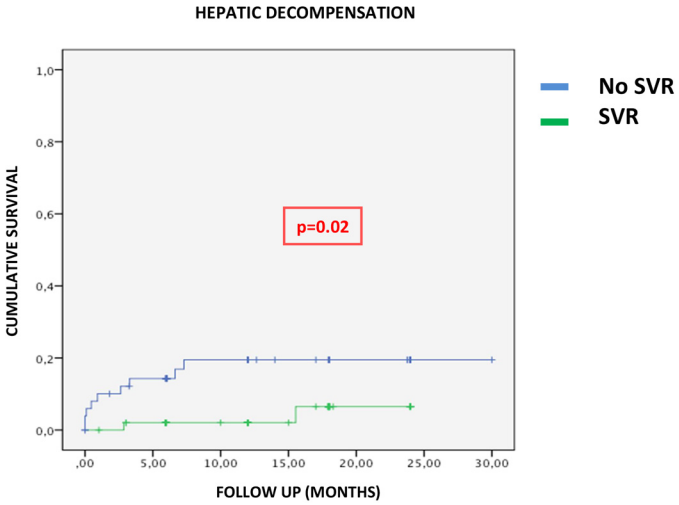
Background and Aims: Antiviral treatment with triple therapy achieved a sustained viral response (SVR12) in a significant number of patients with advanced fibrosis. The aim of this study was to evaluate the development of complications and the evolution of fibrosis after treatment

Methods: Hepatic decompensation (HD) data were collected prospectively until the end of follow up or liver transplantation (LT) and FibroScan® at 6 and 12 months after treatment in two university hospitals.

Results: A total of 226 consecutive patients with SVR12 (59.2%) were included. 112 patients were classified as F4, the baseline characteristics were: Age 64.6 (SD 9.2), ALT 109.5 (SD 64.8), Bilirubin 1.0 (SD 0.6), Albumin 4.2 (SD 0.4), Platelets 138.5×10^9 (SD 50.3×10^9), INR 2.4 (SD 10.7), Fibroscan (kPa) 21.6 (SD 11.6).
The SVR12 was 47.3% (53 patients). 21 patients presented HD (18.8%), of these 10 (8.9%) during treatment and 11 (9.7%) at the end. The most frequent complications were ascites 11 (9.8%) and hepatocarcinoma 8 (7.1%). 8 patients (7.1%) required liver transplantation (LT) and 4 patients (3.5%) died. The mean follow up to the HD or end of follow-up was 12.6 months (SD 7.6). SVR12 patients had lower frequency of HD (4% vs 17.3%; p = 0.03). The cumulative probability of HD year post treatment SVR12 group was significantly lower (2% vs 19.5%; p = 0.02) (Figure 1). In the group of SVR12 the need for LT was significantly lower (0% vs 9.6%; p = 0.03). Were significantly related to the presence of HD the values of albumin [4.3 (SD 0.4) vs 3.7 (SD 0.3); p < 0.001] and fibroscan [20.5 (SD 10.7) vs 32.1 (15.6); p = 0.02]; patients with SVR12 showed a greater decrease of fibrosis in a significantly higher percentage (60% vs 31.6%; p = 0.04).

Conclusions: The SVR12 in patients with advanced liver disease was associated with an improvement in fibrosis stage and a lower rate of decompensation and liver transplantation.



P0813

ALL ORAL THERAPY (SOFOSBUVIR–RIBAVIRIN) COMBINATION IN SEVERE HCV-MIXED CRYOGLOBULINEMIA VASCULITIS, THE VASCUVALDIC STUDY

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Background and Aims: The standard of care treatment of patients with HCV-mixed cryoglobulinemia vasculitis (MC) includes Peg-IFN alpha plus Ribavirin, w/wo Rituximab. Thirty to 40% of patients are non-responders, relapsers or intolerant to such combination. Our aim was to analyze the safety and efficacy of a Sofosbuvir/Ribavirin combination in active severe and/or refractory HCV-MC.

Methods: Open label prospective single-center cohort study including 18 patients with HCV-MC vasculitis. All patients received Sofosbuvir 400 mg/d and Ribavirin (200 to 1200 mg daily), for 24 weeks.

Results: Median age 61.5 years (28–88), 50% women, HCV genotype 1 (n = 12), 2 (n = 2), 4 (n = 3) and 5 (n = 1). Eleven patients (61%) were non-responders to previous antiviral therapy, including Peg-IFN alpha/Ribavirin/Protease inhibitor (n = 6). Three patients had been also previously treated with Rituximab.
At baseline, mean HCV RNA level was 5.9 log copies/mL; Metavir fibrosis score was stage 4 in 8 cases, stage 3 in 2 cases, stage 2 in 5 cases, stage 1 in 1 case and stage 0 in 2 cases. Eleven patients had a type II IgMk MC. Main HCV-MC manifestations included purpura (n = 13), polyneuropathy (n = 9), arthralgia (n = 12), and kidney involvement (n = 3). The median serum MC, C4 and rheumatoid factor levels were 0.17 g/l, 0.10 g/l and 26 IU/ml, respectively.
At week 24 (end of treatment), 14 patients (78%) were complete clinical responders of cryoglobulinemia vasculitis. Negative viremia i.e. <12 IU/ml (at week 24) was evidenced in 13 out of 16 (81%) cases. The cryoglobulin level decreased from 0.17 to 0.05 g/l (p < 0.05) while the C4 serum level increased from 0.10 to 0.15 g/l (p < 0.05). The alanine aminotransferase level dropped from 50 to 17 IU/ml (p < 0.05). Adverse events occurred in 13 patients (72%) and included anemia (n = 6), insomnia (n = 4), asthenia (n = 3), infection (n = 3) (herpes, pneumonia and prostatitis) and nausea (n = 1).

Conclusions: Sofosbuvir plus ribavirin combination is highly effective in active severe and/or refractory HCV-MC with an acceptable safety profile. Virological (SVR12) and clinical responses at week 36 will be presented at the meeting.

P0814

HEPATIC IMPROVEMENT IN RESPONSE TO LEDIPASVIR/SOFOSBUVIR/RIBAVIRIN AS MEASURED BY THE HEPQUANT® (HQ)-SHUNT TEST IN LIVER TRANSPLANT (LT) RECIPIENTS WITH ALLOGRAFT FIBROSIS OR CIRRHOSIS AND NON-LT PATIENTS WITH DECOMPENSATED CIRRHOSIS

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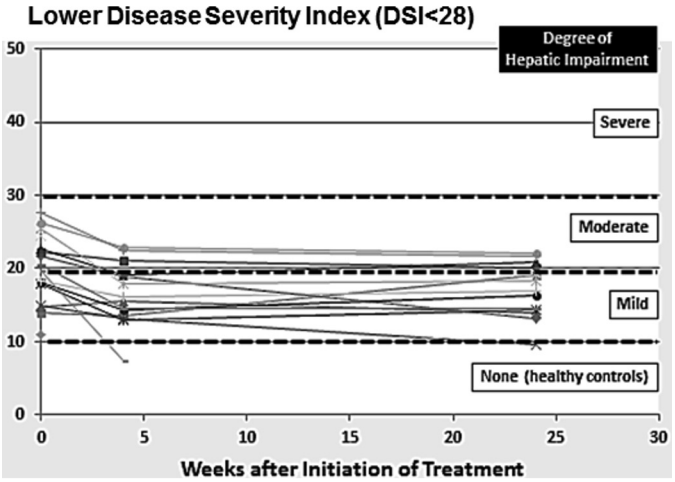
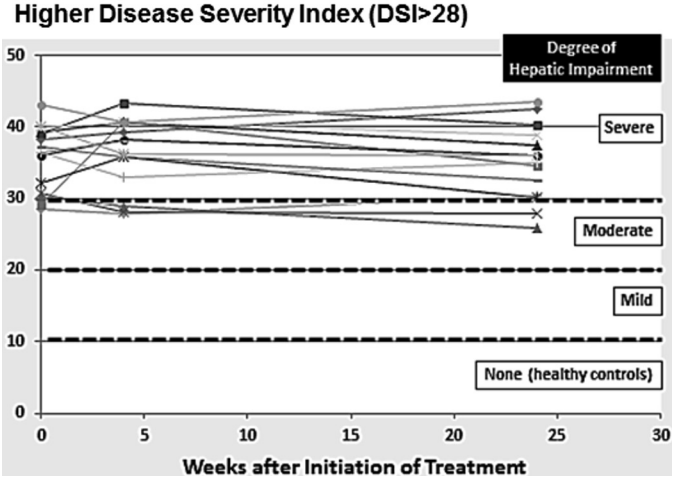
Background and Aims: A primary goal of the treatment of advanced HCV is normalization of hepatic function and the portal circulation. In SOLAR-1, recipients of liver transplantation (LT) with either fibrosis or cirrhosis, and non-LT patients with decompensated cirrhosis were treated with ledipasvir/sofosbuvir/ribavirin

(LDV/SOF/RBV) for 12 or 24 weeks. We assessed changes in hepatic function and the portal circulation during, and after LDV/SOF/RBV using HQ-SHUNT, a test employing dual cholates labeled with stable isotopes and given simultaneously both orally (po) and intravenously (iv). Baseline, Week 4, and Week 24 results were available. Completed results through Week 48 will be presented.

Methods: The study included 31 patients enrolled in SOLAR-1: 10 with LT and F0–F3 fibrosis, 11 with LT and cirrhosis (3 CTP A, 6 CTP B, 2 CTP C) and 10 non-LT patients with decompensated cirrhosis (4 CTP B, 6 CTP C). The HQ-SHUNT test involves serum sampling prior to, and at 5, 20, 45, 60, and 90 minutes after administering the cholates. Clearance of po D4-cholate defines Portal Hepatic Filtration Rate (HFR). Clearance of iv ^{13}C-cholate defines Systemic HFR. The ratio of Systemic to Portal HFR defines SHUNT. HFRs and SHUNT have been modeled in other studies to derive a disease severity index (DSI) predictive of clinical outcomes. A DSI >28 signifies severe hepatic impairment. Healthy controls have DSI ≤10.

Results: At baseline, HFRs were higher and SHUNT and DSI were lower in non-cirrhotic LT recipients compared to cirrhotic LT recipients, and in cirrhotic LT recipients compared to the decompensated non-LT patients. Median baseline DSI was 27.6 – 16 patients had DSI <28 (10 F0–F3, 1 CTP A, 5 CTP B) and 15 had DSI >28 (2 CTP A, 5 CTP B, 8 CTP C). By Week 4, HCV RNA was <LLOQ and patients with baseline DSI <28 had significant drop in DSI. Patients with DSI >28 did not improve. DSI, HFRs and SHUNT did not change between Weeks 4 and 24 in either DSI group. One patient with SBP between Weeks 0 and 4, had marked increase in DSI at Week 4, and decline in DSI by Week 24 (dotted line in right panel).





Conclusions: A DSI cutoff of 28 correlates with severity of liver disease and identifies patients with (DSI <28) and without (DSI >28) early hepatic improvement during LDV/SOF/RBV. Early hepatic improvement is likely related to improvement in hepatocyte perfusion and function. Further hepatic improvement beyond Week 4, as measured by decline in DSI, will likely require resolution of hepatic fibrosis – a much slower natural process.

P0815

COST-EFFECTIVENESS OF TREATING DIFFERENT STAGES OF GENOTYPE 1 HEPATITIS C VIRUS (HCV) WITH ABBVIE 3D (ABT-450/RITONAVIR/OMBITASVIR AND DASABUVIR) +/− RIBAVIRIN COMPARED TO NO TREATMENT IN THE UNITED STATES

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Background and Aims: New interferon (IFN)-free therapies for the treatment of hepatitis C virus (HCV) offer better viral clearance rates and safety profiles than older therapies but are priced higher. As a result, payers have questioned the value of treating all HCV patients with IFN-free therapies and have proposed reserving treatment for patients with more advanced liver disease. The objective of this study was to determine the cost-effectiveness of treating genotype 1 HCV patients with the AbbVie 3D (ABT-450/ritonavir/ombitasvir and dasabuvir) +/− ribavirin regimen compared to no treatment at different fibrosis stages in the United States.

Methods: A Markov model was developed to determine the cost-effectiveness of HCV treatment with AbbVie 3D compared to no treatment. The model population, dosing, and efficacy information were based on the AbbVie 3D phase 3 clinical trials. Disease stage was grouped based on fibrosis score (F0, F1, F2, F3, and F4). A hypothetical cost of AbbVie 3D was assumed to be equal to the cost of sofosbuvir. Effects were measured in quality-adjusted life-years (QALYs) and cost-effectiveness was quantified as an incremental cost-effectiveness ratio (ICER). The ICER was calculated for patients across all stages (F0–F4) and compared to the ICER for treating patients with more severe disease, separately for treatment naïve and experienced patients.

Table 1. Cost-effectiveness of treating different stages of HCV with AbbVie 3D compared to no treatment

Treatment strategy	ICER of AbbVie 3D compared to no treatment
Treatment naive	
Treat F0–F4 patients	$19,193/QALY
Treat F1–F4 patients	$15,701
Treat F2–F4 patients	$12,612
Treat F3–F4 patients	$11,792
Treat F4 patients	$16,181
Treatment experienced	
Treat F0–F4 patients	$18,411/QALY
Treat F1–F4 patients	$16,081
Treat F2–F4 patients	$14,523
Treat F3–F4 patients	$14,519
Treat F4 patients	$16,956

Results: It is cost-effective to treat treatment naïve patients across all stages (F0–F4) at $19,193/QALY. The ICER is more favorable with treatment of F1–F4 patients ($15,701), F2–F4 ($12,612), and F3–F4 ($11,792) but increases if treatment is restricted to only F4 patients ($16,181) (Table 1). Treatment experienced patients also have favorable cost-effectiveness ratios. The ICER for treating F0–F4 treatment experienced patients is $18,411/QALY. Similarly to the treatment naïve group, the ICER is slightly more favorable when restricting treatment to F1–F4 ($16,081), F2–F4 ($14,523), F3–F4

($14,519) but increases if treatment is restricted to only F4 patients ($16,956). Results were robust to variation in model parameters including costs, efficacy, health utilities, discount rates, and fibrosis and disease progression rates.
Conclusions: It is cost-effective to treat HCV patients across all disease stages and treatment histories. All incremental cost-effectiveness ratios are well below the cost per QALY threshold of $50,000.

P0816
REDUCTION IN ANNUAL MEDICAL COSTS WITH EARLY TREATMENT OF HCV USING ABBVIE 3D (ABT-450/RITONAVIR/ OMBITASVIR AND DASABUVIR) +/− RIBAVIRIN IN THE UNITED STATES

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Background and Aims: Treating and curing patients with hepatitis C virus (HCV) is believed to reduce future medical costs; however, the timing and stage at which to treat HCV has been a topic of debate. Early treatment has been associated with greater viral clearance rates while treating HCV patients in later disease stages has been shown to leave patients at excess risk for liver complications including hepatocellular carcinoma and liver transplant. The aim of this study was to determine the average annual lifetime post-treatment medical costs for patients treated at varying stages of liver disease in the United States.
Methods: A Markov model of the natural history of HCV was developed based on previous HCV models. The model population and efficacy rates were based on AbbVie 3D (ABT-450/ritonavir/ombitasvir and dasabuvir) phase 3 clinical trials. Disease stage was classified into 5 groups based on METAVIR score (F0, F1, F2, F3, and F4). Costs were in US dollars and based on a systematic literature review of US costs in HCV. All direct medical costs, with the exception of HCV drug costs, were included in the model. Analyses were run with costs and life years undiscounted and discounted at a rate of 3%. The model was run over a life time horizon with patients entering the model at each fibrosis stage and receiving treatment with AbbVie 3D. Annual medical costs were calculated by dividing lifetime costs by life years. Analyses were run separately for treatment naïve and experienced patients.

Table 1. Average annual lifetime direct medical costs after treatment with AbbVie 3D

Stage of disease at treatment	Average annual lifetime direct medical costs post-treatment	
	Undiscounted	Discounted
Treatment naive		
F0	$314	$228
F1	$441	$320
F2	$597	$445
F3	$806	$633
F4	$3,266	$2,938
Treatment experienced		
F0	$298	$221
F1	$417	$308
F2	$564	$427
F3	$760	$604
F4	$3,128	$2,823

Results: Initiation of treatment at later stages of fibrosis resulted in an increase in average annual lifetime post-treatment costs increase while the life years saved decreased. This result occurred in both treatment naïve and experienced patients. Treatment naïve patients treated in F0 stage had average annual medical costs of $314 ($228 discounted) post-treatment. This value gradually increased for patients in F1, F2, and F3 stages. Patients treated in F4 stage had significantly greater annual medical costs that were over 10-fold greater that annual medical costs for patients treated in F0.
Conclusions: Our model suggests that HCV patients who are treated earlier in the disease process incur lower average annual lifetime post-treatment annual medical costs compared to patients treated later in the disease process. Consistent with existing literature, annual post-treatment medical costs are much higher for patients in F4 stage compared to other stages.

P0817
COST-EFFECTIVENESS OF TREATING CHRONIC HEPATITIS C GENOTYPE-1 WITH LEDIPASVIR AND SOFOSBUVIR IN GERMANY

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Background and Aims: Treatment of chronic hepatitis C has significantly improved with the introduction of recent direct acting antivirals (DAAs) allowing more than 90% of patients to be cured. Increased effectiveness was accompanied by significantly higher treatment costs, especially for interferon-free treatment regimens. The aim of the present study was to analyze the cost-effectiveness of treating patients with the new released DAA combination of ledipasvir/sofosbuvir (LDV/SOF).
Methods: A decision-analytic markov model was used to evaluate the long-term cost-effectiveness of LDV/SOF treatment compared to current available treatment regimens for genotype-1 patients: peginterferon/ribavirin (PR); telaprevir/PR (TVR/PR); boceprevir/PR (BOC/PR); simeprevir/PR (SMV/PR); sofosbuvir/PR (SOF/PR); sofosbuvir/R (SOF/R), sofosbuvir/daclatasvir (SOF/DAC) and SOF/SMV. Analyses were conducted for treatment-naive (TN) patients receiving LDV/SOF for 8 or 12 weeks and treatment-experienced (TE) patients receiving 12 weeks of LDV/SOF(+R). Payers perspective, discount-rate of 3%, lifetime horizon were used and a proportion of 19% cirrhotic patients in both TN and TE patients was assumed. Sustained virological response (SVR) was based on clinical trials. Other model parameters such as transition probabilities, quality-of-life and costs were derived from published literature or public sources.

Table 1. Total costs and QALYs for alternative treatment regimens and ICER of LDV/SOF compared to other treatment regimens

Treatment	Total costs	Total QALYs	ICER
Treatment-naive			
LDV/SOF (8 or 12 weeks)	58,631 €	18.963	reference
No treatment	19,533 €	15.914	12,821 €/QALY
PR (48 weeks)	32,107 €	16.994	13,472€/QALY
BOC/PR (RGT)	49,414 €	17.779	7,285 €/QALY
TVR/PR (RGT)	52,503 €	17.982	6,247 €/QALY
SMV/PR (24 weeks)	66,663 €	18.159	dominated
SOF/PR (12 weeks)	69,787 €	18.611	dominated
SOF/R (24 weeks)	129,764 €	17.589	dominated
DAC/SOF (12 or 24 weeks)	119,412 €	19.065	595,455 €/QALY
SOF/SMV (12 weeks)[a]	110,742 €	18.831	dominated
Treatment-experienced			
LDV/SOF(+R) (12 weeks)	70,449 €	17.720	reference
No treatment	18,448 €	15.052	19,492 €/QALY
SOF/PR (12 weeks)	70,605 €	17.108	dominated
SMV/PR (48 weeks)[b]	75,861 €	16.999	dominated
DAC/SOF (24 weeks)	195,087 €	17.642	910,792 €/QALY
SOF/SMV (12 weeks)[a,b]	110,036 €	17.857	dominated

[a] SMV/SOF should only be used in patients intolerant to or ineligible for interferon therapy; [b] only for P/R failures.

Results: Treating TN patients with LDV/SOF resulted in average lifetime costs of €58,631 while achieving 18.96 QALYs. Incremental cost-effectiveness ratios were €13,472/QALY compared to PR for 48 weeks; €7,285/QALY compared to BOC/PR RGT; €6,247/QALY compared to TVR/PR RGT and €12,821/QALY compared to no treatment. LDV/SOF was more effective and had lower total costs compared to SOF/PR for 12 weeks, SOF/R for 24 weeks, SMV/PR for 24 weeks and SOF/SMV for 12 weeks. Total QALYs in LDV/SOF were slightly lower compared to SOF/DAC treatment, but also resulted in considerably lower costs. Results for TE patients are shown in Table 1.
Conclusions: This analysis showed LDV/SOF treatment is cost-effective considering international thresholds. Considering SVR-rates in current DAAs, model results are mainly influenced by costs for antiviral treatment. Average LDV/SOF treatment costs have been reduced in TN patients and are almost equivalent for TE patients compared to SOF/PR. Outcomes and average treatment costs should be verified in analyses using real-life data since many trials relate on small patient numbers.

P0818
ANALYSIS OF NS3 PROTEASE RESISTANCE-ASSOCIATED VARIANTS AND PHENOTYPES FOR THE PREDICTION OF TREATMENT RESPONSE TO HCV TRIPLE THERAPY

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Background and Aims: Triple therapies with NS3 protease inhibitors (PI) Telaprevir (TVR) or Boceprevir (BOC) were widely used for treatment of HCV (hepatitis C virus) genotype 1 infected patients in the past 3 years and this treatment is still the standard of care in many countries. In the present study, several viral and host factors like pre-existing RAVs, phenotypic drug susceptibility, viral load, viral subtype, cirrhosis, treatment history, IP10 levels (interferon-gamma inducible protein) and *IL28B* genotype were investigated in association with response to triple therapy.
Methods: Patients were enrolled in a prospective multicenter study to analyze baseline resistance (PIRB, n = 211) and resistance after treatment failure (PISA, n = 92) together with clinical parameters. Patient NS3 RAVs were studied at positions V36, T54, V55, V107, R117, R155, A156, V158, V170, M175 by population-based sequencing. For phenotypic resistance characterization, we created subgenomic replicon-based NS3-libraries of patients with virologic failure (n = 30) and a corresponding cohort with SVR (sustained virologic response) (n = 21).
Results: Within 24 weeks after treatment failure, RAVs appeared frequently (62%) with the combined mutation V36M+R155K as most prevalent variant (49%). Investigations on the association of baseline RAVs with phenotypes and clinical parameters were conducted in a group of 41 patients with virologic failure and 115 individuals with SVR. RAVs occurred rarely at baseline [n = 2 (5%), virologic failure; n = 10 (9%), SVR] and correlated not with treatment response. We detected sensitive (n = 28, <1-fold change IC_{50} compared to wildtype replicon) and resistant (n = 23, >1-fold change) phenotypes not correlating with the presence of RAVs. The number of resistant phenotypes was comparable in both groups (48% SVR, 43% virologic failure). Other predictors of treatment response were not (HCV viral load, subtype) or weakly (IL28B, prior null response, cirrhosis, IP10 levels; $P < 0.05$) associated with outcome as single parameters. In combined analyses, we detected a highly significant correlation of a higher number of negative predictors in patients with virologic failure compared to SVR (mean 3.6 versus 2.3, $P < 0.001$).
Conclusions: Failure to first generation PI-based triple therapy is a multifactorial event shown by a lack of correlation with single predictors but with the number of negative predictors including RAVs, viral phenotype, viral load and HCV subtype, prior null response, IL28B genotype, IP10 levels and cirrhosis.

P0819
SIMEPREVIR AND SOFOSBUVIR WITH MODIFIED DOSES OF RIBAVIRIN (RBV) THERAPY ON TELAPREVIR EXPERIENCED CO INFECTED (WITH HIV) CIRRHOTICS WITH CHRONIC HEPATITIS C (CHC). A RANDOMIZED OPEN LABEL CLINICAL PILOT STUDY: STOP C

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Background and Aims: Cirrhotics with CHC still remains a challenge. Co-infected cirrhotics (HIV+CHC) are at a greater risk for rapid decompensation affecting QOL and have a higher transplant risk burden. Interferon based therapy entails a longer duration with an increased susceptibility of infections and marrow suppression warranting use of growth factors and even discontinuation of therapy/treatment failure. Telaprevir; a protease inhibitor (PI) based therapy have proved efficacious in co-infected patients. Newer generation PI coupled with polymerase inhibitors and adjusted doses of RBV have shown favorable outcomes. To evaluate the efficacy of Simeprevir, Sofosbuvir with RBV in prior Telaprevir experienced co-infected cirrhotics.
Methods: Fifty (n = 50) co-infected (HIV+CHC, non AIDS) cirrhotics with mean MELD 16, HIV RNA undetectable, mean CD4 count 439, Hb 10.7, HCV RNA 1.7 million copies, mean platelet count 104, albumin 2.9 and WBC 4600. 18 genotype 1a and 32 genotype 1b.
Exclusion criteria: HBV, decompensated cirrhosis, hemolytic disease, heart failure, AIDS, Alcohol consumption >30 g/day, CrCl <50%, uncontrolled diabetes, portal hypertension, Patients on any herbal medications.
Group A: Simeprevir 150 mg + Sofosbuvir 400 mg + RBV for 24 weeks
Group B: Simeprevir 150 mg + Sofosbuvir 400 mg + RBV 1000 mg for 16 weeks
Results: See the table.

Undetectable	Group A (n = 22)	Group B (n = 28)
48 hours	2/22 (9%)	4/28 (14%)
1 week	3/22 (14%)	7/28 (25%)
4 weeks	16/22 (73%)	19/28 (68%)
8 weeks	17/22 (77%)	22/28 (78%)
12 weeks	17/22 (77%)	23/28 (82%)
16 weeks	17/22 (77%)	23/28 (82%)
24 weeks	18/22 (83%)	
28 weeks		23/28 (82%) [SVR 12]
36 weeks	18/22 (83%) [SVR 12]	
40 weeks	18/22 (83%)	23/28 (82%)

Conclusions: The combination of Interferon free regimen in special population with prior experienced PI demonstrated no difference of SVR in 16th week over 24th weeks. Group A 83% compared to Group B 82% responders were noted. This regimen was well tolerated and has a better safety profile than conventional trials.

P0820

PHARMACOKINETICS OF PARITAPREVIR, OMBITASVIR, DASABUVIR, RITONAVIR AND RIBAVIRIN IN SUBJECTS WITH HCV GENOTYPE 1 INFECTION IN PHASE 3 STUDIES

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Background and Aims: AbbVie currently has 3 direct acting antiviral agents (DAAs) in clinical development for the treatment of HCV genotype 1 (GT1) infection, paritaprevir, an HCV NS3/4A protease inhibitor identified by AbbVie and Enanta as a lead compound for clinical development, (administered with ritonavir, a pharmacokinetic enhancer), ombitasvir, a NS5A inhibitor and dasabuvir a NS5B non-nucleoside polymerase inhibitor. Objectives of these analyses were to characterize the pharmacokinetics of the 3 DAAs, ritonavir and ribavirin (RBV) to identify patient-specific covariates affecting exposure.

Table 1. Covariates evaluated for effect on pharmacokinetics of DAAs, Ritonavir and RBV

Patient-specific covariates
On apparent clearance: age, sex, black race, body weight, body mass index, body surface area, creatinine clearance, non-responders/naïve, genotype 1 a/b, Hispanic/Latino ethnicity, Asian, cirrhosis, use of methadone/buprenorphine, use of ribavirin and comedications (see below for the drug classes and categories).
On apparent volume parameters: age, sex, body weight, body surface area and body mass index.
Drug Classes
Proton pump inhibitors/GI agents (those that interfere with stomach pH, and does not include laxatives)
Opioids
Antidepressants/Anxiolytics/Benzodiazepines/Barbiturates
Antihypertensives
PDE5 inhibitors
Non-opioid analgesics
Statins (HMG Co-A inhibitors) and lipid-lowering agents
Antipsychotics
Antiepileptic drugs (AEDs)/anticonvulsants
Antidiabetics
Antihistamines/antiallergics/respiratory agents
Hormonal contraceptives
Hormonal replacement therapies
Steroids
Anti-infectives
Drug categories: Inhibitors and/or inducers of metabolic enzymes and transporters
CYP 3A4/5/7
CYP 2C8
CYP 2D6
CYP 1A2
CYP 2C9
CYP 2C19
CYP 2B6
UGTs
P-gp
MRP2
BCRP
OATP1B1
OATP1B3

Methods: Pharmacokinetic data of all 3 DAAs ± RBV were obtained for 2348 HCV GT1-infected patients who enrolled in one Phase 2 and six Phase 3 trials (1841 of them received RBV). Population pharmacokinetic models were built using the program NONMEM 7.3. Effect of patient-specific covariates (demographics, compensated cirrhosis Child–Pugh class A, renal impairment, and treatment experience), RBV and 15 comedication drug classes and/or 19 enzyme/transporter inhibitor/inducer categories (see table) on pharmacokinetics were evaluated.

Results: A one-compartment model best described paritaprevir, ritonavir and ombitasvir concentration-time profiles, while a two-compartment model best described dasabuvir and RBV concentration time profiles. Steady-state Cmax (ng/mL) and AUC24 (ng·h/mL) values, respectively, for the reference population included paritaprevir: 97 and 1130; ritonavir: 396 and 3400; ombitasvir: 49 and 857; dasabuvir: 533 and 8150; and RBV: 1450 and 38200. Changes in age (by 10 years) and body weight (by 10 kg) showed minimal impact on exposures (<20% change). Females had higher exposures (15%>100%) of the DAAs and RBV than males. Subjects with cirrhosis had higher paritaprevir (140%) and dasabuvir (40%) exposures, but comparable ombitasvir, RBV and ritonavir exposures as noncirrhotic patients. Mild renal impairment did not affect paritaprevir and ombitasvir, while having a <15% impact on dasabuvir, RBV and ritonavir exposures. Subjects on concomitant anti-diabetics or opioids had up to approximately 50% higher exposures of paritaprevir than without these concomitant medications. None of the other DAAs, ritonavir or ribavirin were influenced by comedications. RBV did not significantly impact DAA or ritonavir pharmacokinetics.

Conclusions: Based on the broad therapeutic range and tolerability of the 3DAA regimen, no dose adjustments of the DAAs are required for the evaluated covariates.

P0821

ARE EXTENDED DURATIONS AND/OR RIBAVIRIN USEFUL FOR GENOTYPE 1 (G1) CIRRHOTIC PATIENTS WHO RECEIVE DAAS COMBINATION? A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS (RCTS)

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Background and Aims: The use of ribavirin and a treatment duration longer than 12 weeks have been suggested to increase SVR12 rates in G1 cirrhotic patients receiving DAAs, especially G1 treatment-experienced (TE) cirrhotic patients. The aim of this meta-analysis was to address this issue.

Methods: All phase II and phase III RCTs published or communicated to date which included GI naïve (TN) or TE cirrhotics and contained arms with or without ribavirin and/or compared 12 weeks vs. extended (i.e., 18 or 24 weeks) treatment durations were included. All DAAs regimen were analyzed together. For TN and TE patients, 2 analyses evaluated the ribavirin use (regardless of the treatment duration and for a fixed 12 weeks treatment duration) and 3 analyses evaluated the treatment duration (regardless of, with, or without ribavirin). Meta-analyses were performed according to the Der Simonian and Laird method and reported weight-adjusted SVR12 gains.

Results: 10 RCTs including 1307 G1 cirrhotic patients (573 TN, 734 TE) were selected for this meta-analysis. The distribution of DAA combinations was as follows: SIM+SOF (COSMOS, N = 168), 3D Abbvie (TURQUOISE II, N = 380), DCV-TRIO BMS (UNITY 2, N = 202), GPV+EBV (C-WORTHY, N = 253), and LDV+SOF (6 RCTs, N = 496). In G1 TN cirrhotic patients, the use of ribavirin (ΔSVR = +2.45%; 95% CI: −1.27% to +6.16%, NS, N = 411) or an extended duration (ΔSVR = +0.64%; 95% CI: −3.03% to +4.32%, NS, N = 434) did not increase SVR12. In G1 TE cirrhotic patients, the use of ribavirin did not increase SVR12 (ΔSVR = −0.04%; 95% CI: −3.84% to +3.74%, NS, N = 503), even in analyses restricted to 12 wks treatment duration (ΔSVR = +1.77%; 95% CI: −3.85% to +7.38%, NS, N = 267). Conversely, extended duration was associated with higher SVR rates (ΔSVR = +7.98%; 95% CI: +1.26 to +14.70%, p = 0.019, N = 539), even with ribavirin use (ΔSVR = +12.87%; 95% CI: +0.39 to +25.35%,

p = 0.008, N = 280). The magnitude of SVR gain was similar between the different DAA regimen but was higher in studies incorporating patients who experienced failure to previous PI+P/R regimen (+9.17% vs. +6.90%).

Conclusions: The use of ribavirin does not increase SVR12 rates in TN and TE G1 cirrhotic patients. In TN G1 cirrhotic patients, treatment durations >12 weeks are not useful. In TE G1 cirrhotic patients, treatment durations >12 weeks increase SVR12 rates by 8% and thus should be recommended.

P0822

SAFETY AND EFFICACY OF SOFOSBUVIR CONTAINING REGIMENS FOR HEPATITIS C: COMMUNITY TREATMENT OF A REAL WORLD POPULATION WITH ADVANCED LIVER FIBROSIS

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Background and Aims: The use of direct-acting antivirals (DAAs) such as sofosbuvir (SOF), simeprevir (SIM) and daclatasvir (DAC) created a major paradigm shift in the treatment of chronic hepatitis C. The aim of this study was to evaluate the safety and efficacy of DAAs utilized in clinical practice in experienced patients with advanced liver fibrosis

Methods: AVD LIB is a longitudinal observational study of patients treated with DDAs at community medical centers (n = 13). Demographic, clinical, adverse events and virological data were collected during treatment and post treatment follow-up.

Results: Since March 2014, 209 patients have started treatment according to restrictive French guidelines (interferon based regimen failure and/or fibrosis F3 or F4) and were included in this study. Mean age was 59 years, 58% male. Genotype 1 was seen in 167 patients (71%), genotype 2 in 4 (2%), genotype 3 in 34 (16%) genotype 4 in 24 (11%). Comorbidities included diabetes in 10% and anxiety and depression in 15%. Viral load >800,000 was seen in 70%, 21% were treatment naïve and 79% have failed on interferon bases regimen including patients with 1 generation protease inhibitors failure. Cirrhosis was present in 66% (Child A in 91%, B in 9% of cases).

Treatment regimens: 12 weeks regimen for genotype 1 included PEG + RBV + SOF in 23 (16%), SOF + SIM +/− RBV in 47 (32%), SOF + DAC in 11 (7%); 24 weeks regimens for genotype 1 included SOF + RBV in 11 (7%) and SOF + DAC +/− RBV in 53 (36%). 12 weeks regimen of PEG + RBV + SOF and of SOF + RBV were used in respectively 2 patients with genotype 2. Among patients with genotype 3, 8 (24%) received 12 weeks of PEG + RBV + SOF, 8 (24%) 24 weeks of SOF + RBV and 18 (53%) 24 weeks of SOF + DAC. Among patients with genotype 4, 4 (17%) received 12 weeks of PEG + RBV + SOF, 12 (50%) 12 weeks of SOF + SIM and 8 (33%) 24 weeks of SOF + DAC +/− RBV.

Any serious adverse event was reported, the most common side effect was asthenia (26 patients). Normal life with any side effects was seen in 37% of patients. At week 4, HCV RNA was undetected in 93% of patients. SVR12 weeks after treatment was available in 29 patients who received 12 weeks of PEG + RBV + SOF. Relapse occurred in 8 of these patients. The SVR12 rate in experienced patients receiving PEG + RBV + SOF was 72%.

Conclusions: An examination of real life advanced hepatitis C in community medical practice: AVD LIB study is underway and SVR and safety data will be available at the meeting

P0823

PHARMACOKINETICS OF PARITAPREVIR, OMBITASVIR, RITONAVIR AND RIBAVIRIN IN SUBJECTS WITH HCV GENOTYPE 4 INFECTION

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Background and Aims: Paritaprevir is an HCV NS3/4A protease inhibitor identified by AbbVie and Enanta as a lead compound for clinical development, [administered with ritonavir (RTV), a pharmacokinetic (PK) enhancer] and ombitasvir is a NS5A inhibitor. A recently completed study evaluated these two direct acting antivirals (DAA) in combination (2DAA), with and without ribavirin (RBV) in non-cirrhotic patients with HCV Genotype 4 (GT4) infection. SVR rates of 91% (40/44) and 100% (91/91) were observed in the RBV-free and RBV-containing regimens respectively. No patient discontinued due to adverse events. Objectives of these analyses were to characterize the pharmacokinetics of the two DAAs, RTV and RBV to identify patient-specific covariates affecting exposure in GT4 infected subjects.

Methods: PK data were obtained from 135 HCV GT4-infected patients without cirrhosis who received 2DAA with RBV (N = 91) or without (N = 44) in a Phase 2b trial. Population PK models were built using the program NONMEM 7.3. Effect of patient-specific covariates (demographics, creatinine clearance and treatment experience), and the coadministration with RBV on pharmacokinetics were evaluated.

Results: A one-compartment model best described paritaprevir and RTV concentration-time profiles, while a two-compartment model best described ombitasvir and RBV concentration-time profiles. Age, weight, sex, prior treatment experience and creatinine clearance did not affect paritaprevir and RTV exposures. Female subjects had 15–48% higher ombitasvir exposures than male subjects, while subjects with mild renal impairment (creatinine clearance 75–105 mL/min) had 8–25% higher ombitasvir exposures than subjects with normal renal function (creatinine clearance 105 mL/min). Renal impairment also affected RBV exposures as subjects with mild renal impairment had 8% higher exposure compared to subjects with normal renal function. RBV did not significantly impact DAA or RTV PK.

Conclusions: Based on the broad therapeutic range and tolerability of the 2DAA regimen, no dose adjustments of the DAAs are required based on the evaluated covariates.

Table 1. Model-predicted steady-state exposure [a] at normal renal function [b]

	AUC_{ss} (ng·h/mL)	$C_{max,ss}$ (ng/mL)	$C_{min,ss}$ (ng/mL)
Paritaprevir	2210	189	13.2
Ombitasvir	1080–1340	72.2–83.1	17.4–25.7
Ritonavir	3360	280	21.8
Ribavirin	18600	1690	1330

[a] Calculated over 24 hours steady state profiles for paritaprevir, ombitasvir, RTV QD regimens and over 12 hours for RBV BID regimen.
[b] Creatinine clearance of 105 mL/min.

P0824

A PHASE 1 STUDY TO EVALUATE THE INTERACTION OF HCV NS5B INHIBITOR MK-3682 WITH HCV NS3/4A PROTEASE INHIBITOR MK-5172 AND HCV NS5A INHIBITOR MK-8408 IN HEALTHY SUBJECTS

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Background and Aims: MK-3682 is a potent and selective pan-genotypic uridine nucleoside monophosphate prodrug inhibitor of the HCV NS5B RNA polymerase being developed with MK-5172, a potent and selective HCV NS3/4A protease inhibitor and MK-8408, a novel HCV NS5A inhibitor, as potential components of a once-daily (QD) all-oral direct-acting antiviral regimen for the treatment of chronic HCV infection. This study evaluated the safety and PK of coadministration of MK-3682 with MK-5172 and MK-8408.
Methods: This was a 3-period, fixed-sequence, open-label drug-interaction study in 18 healthy subjects. In period 1, subjects received MK-5172 200 mg and MK-8408 60 mg QD on days 1–7 followed by a washout on days 8–14. In period 2, subjects received MK-3682 300 mg QD on days 15–21 followed immediately by period 3, in which subjects received MK-3682 300 mg, MK-5172 200 mg and MK-8408 60 mg QD on days 22–28. Blood samples were collected for MK-3682 and its circulating metabolites IDX20664 and IDX23267, MK-5172 and MK-8408. PK parameters were calculated by non-compartmental analysis. Geometric mean ratios (GMR) and 90% confidence intervals (CI) were calculated from the log transformed AUC0–24 and Cmax for all analytes using mixed effect modeling. Safety assessments included ECGs, vital signs, clinical laboratory tests, physical examination and adverse event monitoring.
Results: 18 healthy male and female subjects were enrolled and 17 completed the study. Coadministration with MK-5172 and MK-8408 modestly increased MK-3682 exposures with AUC0–24 and Cmax GMRs [90%CI] of 1.27 [1.16–1.39] and 1.21 [1.03–1.42] and decreased IDX20664 exposures with AUC0–24 and Cmax GMRs [90% CI] of 0.85 [0.81–0.89] and 0.75 [0.72–0.79]. IDX23267 exposures were not affected by MK-5172 and MK-8408. MK-3682 increased MK-5172 exposures but not to a clinically meaningful degree (AUC0–24 and Cmax GMRs [90% CI] of 1.36 [1.11–1.68] and 1.56 [1.19–2.03]) and did not affect MK-8408 exposures (AUC_{0-24} and C_{max} GMRs [90% CI] of 0.99 [0.94–1.04] and 0.99 [0.92–1.06]). Coadministration of MK-3682 with MK-5172 and MK-8408 was well tolerated. One subject was discontinued for a non-drug-related Grade 1 elevation in direct bilirubin.
Conclusions: Coadministration of MK-3682 with MK-5172 and MK-8408 in healthy subjects did not result in clinically significant drug interactions. These results suggest that no dose adjustments of MK-3682, MK-5172 or MK-8408 are needed for coadministration and support further studies of this regimen in HCV patients.

P0825

VARYING EFFICACY OF SOFOSBUVIR TREATMENT REGIMENS IN REAL LIFE SETTINGS

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Background and Aims: The efficacy and safety of Sofosbuvir (SOF) in HCV treatment has been demonstrated in several clinical trials. Nevertheless, few studies have examined its effectiveness in clinical settings. The aim of this study therefore was to evaluate the impact of SOF on treatment outcomes in real life settings.
Methods: We prospectively assessed all genotype 1 (a,b) HCV infected patients attending our clinic who were receiving SOF-based treatments. According with our mDOT model of care, during their treatment period patients were followed each week at the clinic and had a consultation with a nurse, physician and pharmacist; and they had lab tests. Patients enrolled in RCTs were excluded. The primary outcome was sustained virologic response (SVR) at 12 weeks post-treatment by intent-to-treat analysis. Comparisons among treatment groups were assessed by Chi square.
Results: 56 patients were included. They were mainly male (71%), treatment naïve (57%), their mean age was 54 y (IQR 49–58) at treatment initiation, 86% were infected by HCV genotype 1a and 14% by genotype 1b. More than half of patients (64%) were cirrhotic at baseline and 29% were co-infected with HIV. Patients were receiving 3 types of SOF-based regimens: Simeprevir (SIM) + SOF (61%); followed by SOF+PEGINF+Riba (30%); and SOF+Riba (9%). The majority of treatment regimens were 12 and 24 weeks long (91% and 7% respectively) with only 1 patient undergoing a 48 week treatment. 17 patients completed their treatment period and 12 weeks post treatment FU. 16/17 (94%) completed treatment and had undetectable viral loads at the end of treatment, and one died from decompensated liver cirrhosis. However, 12/17 (71% ITT analysis) achieved SVR 12 weeks post-treatment. The rate of SVR varied by regimen, treatment status at baseline and by presence of cirrhosis. SVR was 89% with SIM+SOF, 25% with SOF+Riba and 75% with SOF+Riba+PEGINF ($p = 0.07$). SVR was 100% (3/3) for relapsers; 78% (7/9) for naïve patients and 40% (2/5) for nulls responders ($p = 0.11$). 8/13 (62%) of patients with cirrhosis and 4/4 (100%) without cirrhosis had a SVR ($p = 0.21$). Patients on SIM+SOF were more likely to achieve SVR even after controlling for cirrhosis ($p = 0.08$). No patient had been lost to FU during the study period.
Conclusions: In real life settings, SOF offers a variable rate of SVR. The combination SIM+SOF was the most effective HCV treatment, including for patients with cirrhosis. An mDOT model of care, and close FU, could assure a good adherence to medication.

P0826

EFFECTIVENESS OF SIMEPREVIR (SMV)-CONTAINING REGIMENS AMONG PATIENTS WITH CHRONIC HEPATITIS C VIRUS (HCV) IN VARIOUS US PRACTICE SETTINGS: INTERIM ANALYSIS OF THE SONET STUDY

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Background and Aims: SMV is an oral, once-daily, HCV NS3/4A protease inhibitor approved in various countries for the treatment of chronic HCV infection as part of combination antiviral therapy. Since efficacy and safety in real world practice may vary from clinical trials, SONET evaluates effectiveness of SMV-containing regimens.
Methods: This ongoing, observational, US-based study (N = 300) includes patients aged ≥18 years with chronic HCV G1 infection and a healthcare provider (HCP) decision to treat with SMV; patients with prior direct-acting antiviral use were excluded. Treatment decisions and clinical management were at HCP discretion. The primary endpoint was sustained virologic response (SVR12); secondary endpoints included patient characteristics, prognostic factors of virologic response, SVR4, virologic failure and safety. This planned interim analysis (IA) was conducted when ≥100 patients had Week 4 on-treatment HCV RNA results.

Table 1. IA outcomes

	SMV/SOF (n = 152)	SMV/SOF/RBV (n = 10)	SMV/PegIFN/RBV (n = 6)
HCV RNA outcomes			
RVR	57.7% (56/97)	55.6% (5/9)	100% (5/5)
EOT	100% (38/38)	100% (6/6)	–
SVR4	91.3% (21/23)	100% (1/1)	–
Viral breakthrough	0.7% (1/152)	0	0
Relapse	0.7% (1/152)	0	0
Treatment status			
Discontinuation of all HCV treatment	2.0% (3/152)	10.0% (1/10)	16.7% (1/6)

EOT, end of treatment; PegIFN/RBV, pegylated interferon plus ribavirin; RBV, ribavirin; RVR, rapid virologic response at week 4; SMV, simeprevir; SOF, sofosbuvir; SVR4, sustained virologic response 4 weeks after treatment end.
–, Data not available.

Results: This IA included 168 patients (intent-to-treat population) receiving care in academic centers (15.5%; n = 26), private practice (75.6%; n = 127), and/or integrated health networks (13.1%; n = 22). Median age=58.0 years; 58.9% (99/168) male; 58.9% (99/168) white; 36.3% (61/168) black/African-American; 13.7% (23/168) Hispanic/Latino. HCV G1a and G1b comprised 81.0% (119/147) and 19.0% (28/147) of the population, respectively. At baseline, 16.0% (26/163) of patients reported active alcohol use; 25.3% (42/166) reported a household income of <$10,000 USD. Approximately 29% (49/168) were treatment-experienced; 11.9% (20/168) were prior null-responders; 2.4% (4/168) were prior partial responders. Cirrhosis was reported in 41.0% (68/166) of patients; 13.8% (23/167) had documented varices, 7.2% (12/167) ascites, and 4.8% (8/167) hepatic encephalopathy. Overall, 90.5% (152/168) received SMV+sofosbuvir (SOF), 6.0% (10/168) received SMV+SOF+ribavirin (RBV) and 3.6% (6/168) received SMV+peginterferon (PegIFN)+RBV. At IA, 47 patients completed therapy and 8 patients discontinued ≥1 medication. Table 1 shows outcomes by treatment regimen. At IA, the most common (>5% patients) adverse events (AEs) were headache, nausea and rash; 4.2% (7/168) had serious AEs.
Conclusions: In this IA, SMV+SOF was the most common regimen; of the patients who had this regimen and evaluable SVR4 results, 91.3% achieved SVR4. SVR 12 rates, duration of therapy, outcomes by subgroups (e.g. cirrhotics), and additional safety information will be presented at the meeting.

P0827
A DESCRIPTIVE ANALYSIS OF A REAL-WORLD POPULATION WITH CHRONIC HEPATITIS C (CHC) TREATED WITH SIMEPREVIR (SMV)- AND/OR SOFOSBUVIR (SOF)-BASED REGIMENS: FINDINGS FROM A US PAYER DATABASE

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Background and Aims: Real-world evaluations of newer direct-acting antivirals (DAAs) in CHC patients are needed from large US payer perspectives.
Methods: Medical and pharmacy claims linked to lab data from the Humana Database were analyzed for Medicare Advantage or commercially-insured adults with ≥2 CHC claims (ICD-9 070.44; 070.54) who received therapy containing SMV and/or SOF through 6/2014; those with HIV were excluded. Patients were grouped based on most common regimens in the data: SMV/SOF, SMV/SOF/ribavirin (RBV), SOF/RBV, or SOF/interferon (IFN)/RBV; <3% received other regimens. At time of analysis, baseline (BL) demographics, selected comorbidities and clinical characteristics (e.g., claims-based cirrhosis or end stage liver disease [ESLD], FIB-4 scores) were described and post-treatment follow-up time measured. Methods to control for treatment selection bias were not performed, and comparative analyses were not conducted.

Table: Pre-treatment comorbidities and disease severity

	SMV/SOF (n = 184)	SMV/SOF/RBV (n = 37)	SOF/RBV (n = 269)	SOF/IFN/RBV (n = 225)
Select comorbidities:				
Substance abuse	20%	32%	25%	30%
Anemia	22%	22%	16%	12%
Psychiatric disease	21%	27%	25%	22%
Depression	21%	16%	25%	25%
Diabetes	28%	27%	25%	26%
COPD	14%	16%	19%	22%
Liver disease severity:				
Non-cirrhotic	25%	35%	55%	64%
Cirrhotic	27%	27%	17%	24%
ESLD	48%	38%	27%	12%

COPD, Chronic obstructive pulmonary disease.

Results: There were 715 CHC patients who received therapy with SMV/SOF (n = 184), SMV/SOF/RBV (n = 37), SOF/RBV (n = 269) or SOF/IFN/RBV (n = 225); mean age for cohorts was between 60–62 years; 58%, 68%, 62% and 70% were male; most (85%, 78%, 81% and 80%) had Medicare. BL cirrhosis and ESLD were evaluated as well as selected comorbidities such as substance abuse, anemia, psychiatric disease, depression, diabetes, and chronic obstructive pulmonary disease (Table). Slightly over half in each cohort had calculable FIB-4 scores, of which, 56%, 54%, 34% and 35%, respectively, had scores >3.25. Among those with genotype data, 100% (78/78) SMV/SOF, 94.7% (18/19) SMV/SOF/RBV, 24.4% (29/119) SOF/RBV and 95.8% (92/96) SOF/IFN/RBV were genotype 1. Using prior claims history, 10%, 19%, 12% and 17% of respective cohorts were treatment-experienced, of which 74%, 71%, 28% and 79% had protease inhibitor/IFN/RBV therapy. At time of analysis, less than half of each cohort had post-treatment data ≥1 week.
Conclusions: This analysis of CHC patients predominantly insured through Medicare found that the majority of those who received SMV/SOF±RBV had either cirrhosis or ESLD claims prior to therapy and, using lab data, over half had FIB-4 scores >3.25. These results along with findings on BL comorbidities and prior treatment history offer insight into the real-world characteristics of CHC patients treated with newer DAAs from a US payer perspective. Further analysis with additional follow-up data will provide insights into post-treatment outcomes.
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P0828
RELAPSERS TO NON-INTERFERON, SOVALDI BASED TREATMENT OF G1 CHRONIC HCV WERE INADEQUATELY TREATED

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Background and Aims: Treatment duration can be an important factor in cure of HCV1 and recent studies have shown that longer treatment for 6 months is required for some patients when using Harvoni (Sovaldi + Ledepisvir) and this may also be true of Sovaldi (S) and Olysio (O). We have applied the treatment duration criteria for Harvoni to HCV1 patients treated with the combination of Sovaldi (S) and Olysio (O) in a single community practice since January 2014.
Methods: *Patients:* 35 G1 patients were started on treatment since January 2014. The SVR rate of the 35 patients reaching 4 Weeks (SVR4) and 24 patients at 12 weeks (SVR12) is reported. G1a was present in 19, G1b 14, and G1 2. The response to the last pegasys (P) based treatment (NR non-response, R relapse) with double therapy (P+R) (DT) and triple therapy (P+R+DAA) (TT) was assessed. IL28B CC and cirrhosis was assessed. Harvoni Criteria for treatment duration

was applied to this population: 2 months – naïve non-cirrhotic, RNA <6M IU/ml; 3 months – naïve cirrhosis or non-cirrhosis with >6M IU/ml; 6 months – cirrhosis, treatment experienced.
Results: The overall SVR4 was 91.4% and SVR12 83.3%. IL28B-CC was present in 14 patients (SVR4 100%), G1b in 14 patients (SVR4 100%), 14 naïve patients (SVR4 92.9%) and Q80K in 6/28 patients tested (SVR4 75%). 5 patients had cirrhosis with 1 of these having recurrent HCV with ascites after liver transplant (SVR 4 and 12 40%). The poster table shows the treatment duration recommended if Harvoni had been used compared to the actual 3 months treatment duration with S+O in all patients. Regardless of any other factor, those patients meeting criteria for 2–3 mo treatment duration had a 100% SVR4 and 94% SVR12. All relapse patients had combinations of cirrhosis (4/4), Q80K positive (2/4), and/or prior non-response to DT and TT (3/4).
Conclusions: Based on Harvoni treatment duration criteria, patients qualifying for 2–3 mo treatment had an SVR4 of 100% and SVR12 of 95% with S+O comparable to that expected with Harvoni. The 3/4 patients who relapsed would have qualified for 6 months of Harvoni treatment suggesting that most relapse patients were inadequately treated.

P0829

ON-TREATMENT HCV RNA AS A PREDICTOR OF SUSTAINED VIROLOGIC RESPONSE IN HCV GENOTYPE 3 INFECTED PATIENTS TREATED WITH DACLATASVIR AND SOFOSBUVIR: ANALYSIS OF PHASE 3 ALLY-3 STUDY

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Background and Aims: The use of response-guided therapy (RGT) with some peginterferon/ribavirin-based HCV regimens has improved outcomes for GT-1 treatment, but also complicates patient management. Here, we investigate the utility of RGT in HCV GT-3-infected patients by analysing data from the Phase 3 ALLY-3 Study, in which a 12-week combination of daclatasvir (DCV; NS5A inhibitor) and sofosbuvir (SOF; NS5B polymerase inhibitor) achieved SVR12 rates of 90% and 86% in HCV GT3-infected treatment-naive and -experienced patients, respectively.
Methods: This retrospective analysis included 152 HCV GT3-infected patients who were treatment-naive (N = 101, 66%) or -experienced (N = 51, 34%) and received open-label DCV 60 mg + SOF 400 mg QD for 12 weeks (ALLY-3). Serum HCV RNA levels were quantified using the COBAS Taqman v2.0 (LLOQ=25 IU/mL). Positive and negative predictive values (PPVs and NPVs, respectively) were calculated at Weeks 1, 2, 4, 6, and end of treatment (Week 12) based on HCV RNA
Results: Early decreases in HCV RNA were observed on-treatment, with 34%, 74%, and 95% of total patients having HCV RNA <LLOQ by treatment Week 1, 2, and 4 respectively. The early on-treatment response of <LLOQ at Week 4 in patients with cirrhosis was 91% (29/32). PPVs and NPVs are shown for the total population at Weeks 1, 2, and 4 (Table). As demonstrated by the high PPVs, there was strong concordance between those achieving undetectable HCV RNA at early timepoints and subsequent achievement of SVR12. More than 90% of patients with undetectable HCV RNA at Weeks 1, 2, and/or 4 achieved SVR12. In contrast, as demonstrated by the low NPVs, patients failing to achieve undetectable HCV RNA at early timepoints were still likely to achieve SVR12. Similar results were observed when patients were analysed based on treatment-naive or -experienced status.
Conclusions: Achieving early virologic milestones was associated with a high probability of achieving SVR12 whereas failure to achieve early virologic milestones did not appear to impact outcome. These data do not support the use of early futility rules such as achievement of rapid virologic response to inform on-treatment decisions in HCV GT3-infected patients receiving DCV+SOF.

Table: PPV and NPV data for the total population (N = 152)

	SVR12, n/N (%)	
	PPV	NPV
Week 1		
TND [a]	13/13 (100)	17/139 (12.2)
<LLOQ [b]	49/52 (94.2)	14/100 (14.0)
Week 2		
TND [a]	48/50 (96.0)	15/102 (14.7)
<LLOQ [b]	104/113 (92.0)	8/39 (20.5)
Week 4		
TND [a]	92/101 (91.1)	8/51 (15.7)
<LLOQ [b]	130/145 (89.7)	2/7 (28.6)

PPV: proportion of SVR12 responders among those who responded at times shown.
NPV: proportion of SVR12 failures (non-SVR12) among non-responders at times shown.
[a] <LLOQ, TND.
[b] <LLOQ, TD or TND.

P0830

EARLY HCV KINETICS DURING DUAL ORAL THERAPY WITH DACLATASVIR AND ASUNAPREVIR

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Background and Aims: The dual oral therapy with the first-in-class NS5A replication complex inhibitor Daclatasvir (DCV) and the potent NS3 protease inhibitor Asunaprevir (ASV) has recently approved for the treatment of chronic hepatitis C genotype 1 patients. Previous trials for the treatment was shown promising results for improving SVR to more than 80% in patients with HCV genotype 1b. We conducted a prospective, multicenter study to investigate the effectiveness and safety of the dual oral therapy with DVC and ASV and evaluate the early HCV kinetics during the treatment.
Methods: The patients with HCV genotype 1b have been treated with DCV and ASV since Sep. 2014 (n = 206). The enrolled population was generally older (median 69 years old) that was consistent with HCV epidemiology in Japan and predominantly female (63%). HCV genotype and IL-28B polymorphisms were determined by PCR amplification and sequencing. HCV resistant associated polymorphisms were analyzed by direct sequence of the HCV NS3 and NS5A domains.
Results: HCV-RNA declined rapidly after the initiation of the DCV + ASV treatment, and the reduction of HCV-RNA was comparable with the peg-IFN + RBV + Telaprevir or Simeprevir treatment. HCV-RNA was lower than detection sensitivity limit in 71% of the patients after 2 weeks and 78% of patients achieved RVR. The viral response of HCV was not affected by age, sex, liver fibrosis, previous treatment response, or IL-28B SNPs. Most of the patients enrolled for the study had no resistance-associated

polymorphism in NS3 or NS5A, while two patients with the polymorphism experienced viral breakthrough. Except for the two patients, patients with NS5A Y93H/N or L31M showed similar viral response until 4 weeks of the therapy. The frequently reported adverse events were mild headache, fever, or elevations of ALT, g-GTP or ALP and one patient stopped the therapy because of the onset of brain infarction. There were no further serious adverse events during early course of the treatment.

Conclusions: The response of HCV-RNA was favorable enough during the DCV + ASV therapy, even for women, aged patients, patients with liver cirrhosis or patients with intractable IL-28B polymorphism. However, resistance-associated polymorphism of HCV could affect treatment response, and further examination for absolute quantity of the resistance-associated polymorphism is desired to evaluate the effectiveness for the DCV + ASV treatment.

P0831

SAFETY AND EFFICACY OF SOFOSBUVIR-BASED TREATMENT REGIMENS FOR CHRONIC HEPATITIS C VIRUS INFECTION: A "REAL-LIFE", SINGLE-CENTER EXPERIENCE IN 117 PATIENTS

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Background and Aims: In clinical phase III trials sofosbuvir (SOF)-based treatment regimens in chronic HCV infections achieve high rates of sustained virological response (SVR). The safety profile of SOF seems to be favourable. There are only limited "real-life" data on safety and efficacy of SOF-based therapy in unselected patients outside clinical trials.

Methods: Every patient who started SOF-based therapy [with or without peginterferon (IFN), ribavirin (RBV), simeprevir (SIM) and/or daclatasvir (DAC)] between 01/2014 and 10/2014 was included in the study. Demographical, clinical, laboratory and virological data were collected throughout the entire treatment period. Severe adverse events (SAE) were defined as any grade 3 or 4 laboratory events or any condition leading to prolonged invalidity, hospitalization or death. Potential predictors for SAE and SVR12 were analysed by Fisher's exact test, t-test or rank sum test.

Results: We included 117 patients in our study, of which 60 were infected with genotype (GT) 1 (51.3%), 11 with GT2 (9.4%), 24 with GT3 (20.5%), 20 with GT4 (17.1%), and each 1 with GT5 or GT6 (0.9%, each). In total, 62 patients received IFN/RBV/SOF (53.0%), 18 patients SOF/RBV (15.4%), 12 patients SOF/SIM (10.3%) and 25 patients SOF/DAC±RBV (21.4%). Cirrhosis was diagnosed in 65 patients (55.6%; 25 cirrhotic patients were treated with IFN/RBV/SOF, 8 with SOF/RBV, 12 with SOF/SIM and 20 with SOF/DAC±RBV). 20 SAE occurred in 15 patients (12.8%). No fatal outcome was reported. Use of IFN was no risk factor for SAE (P = 0.219). Age (P = 0.049), MELD score (P = 0.001), cirrhosis (29.3% vs. 6.8%; P = 0.009), baseline thrombocytopenia <100 billion/l (46.7% vs. 11.4%; P = 0.004) and low baseline serum albumin <35 g/l (36.8% vs. 12.1%; P = 0.035) were identified as risk factors for SAE. Non-GT1 infected patients receiving IFN/RBV/SOF achieved SVR12 more often than GT1 patients with IFN/RBV/SOF [20/22 (90.9%) vs. 15/24 (62.5%); P = 0.038]. On-treatment safety data of the entire cohort and SVR12 data of all patients treated with IFN/RBV/SOF, SOF/RBV or SOF/SIM will be available in April 2015.

Conclusions: SOF-based treatment regimens are generally safe and well tolerated. Risk for SAE is not necessarily higher in patients treated with IFN/RBV/SOF compared to patients (with generally more advanced cirrhosis) receiving IFN-free therapy. According to our "real-life" data, IFN/RBV/SOF will remain a reasonable treatment option for patients infected with HCV GT3 or GT4.

P0833

SIMEPREVIR- AND TELAPREVIR-BASED TRIPLE THERAPIES FOR GENOTYPE 1B CHRONIC HEPATITIS C PATIENTS AGED 70 AND OVER IN A MULTICENTRE COHORT STUDY

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Background and Aims: Increasing age has been associated with developing advanced liver fibrosis and hepatocellular carcinoma for chronic hepatitis C. The aim of this study was to evaluate the efficacy analysis in patients aged ≥70 treated with simeprevir and telaprevir-based triple therapies in a prospective, multicenter cohort study.

Methods: This prospective, multicenter study has enrolled 885 genotype 1b patients with chronic hepatitis C who received 12-week triple therapy that included simeprevir (n = 345) or telaprevir (n = 540) combined with pegylated interferon α2b (PEG-IFNα2b) and ribavirin (RBV) followed by a 12-week dual therapy that included PEG-IFNα2b and RBV. A total of 144 patients aged ≥70 were analyzed, 74 and 70 receiving simeprevir and telaprevir-based therapies, respectively. Serum HCV RNA level was measured by COBAS TaqMan HCV test. The undetectable HCV RNA at week 4 (RVR) and sustained virological response at 12 weeks after therapy (SVR_{12W}) was done based on intention-treat-analysis.

Results: The overall rates of RVR and SVR_{12W} were 76.4% and 77.8%, respectively. Of the patients with RVR, 83.6% achieved an SVR_{12W}. While significant differences in RVR rates were found between simeprevir- and telaprevir-based triple therapies (83.8% vs. 68.6%, P = 0.031), SVR rates did not differ between the two therapies (79.7% vs. 75.7%, P = 0.562). The overall treatment discontinuation was 17.4%, but no significant differences were found between the two therapies (14.9% vs. 20%, P = 0.416). The SVR_{12W} rate for interleukin 28B (IL28B) (rs8099917) TT (86.7%) was significantly higher than for IL28B TG/GG (36.9%) (P < 0.001). The rate for no or mild advanced fibrosis (F0–2) (87.3%) was significantly higher than for advanced fibrosis (F3–4) (48.1%) (P < 0.001). The rate for no treatment discontinuation (84.9%) was significantly higher than for treatment discontinuation (44.0%) (P < 0.001). Multivariate analysis extracted IL28B TT (Odds ratio 7.3, P = 0.015), no or mild advanced fibrosis (Odds ratio 12.7. P = 0.003), and no treatment discontinuation (Odds ratio 21.4, P = 0.004) as independent factors associated with SVR_{12W}, but did not RVR. No episode of death or hepatic decompensation was observed.

Conclusions: Simeprevir- and telaprevir-based triple therapies can be used successfully and safely to treat elderly patients with genotype 1b chronic hepatitis C. Treatment discontinuation indicates low effectiveness in these elderly patients.

P0834

SIGNIFICANT DRUG–DRUG INTERACTION BETWEEN SIMEPREVIR AND CYCLOSPORINE A BUT NOT TACROLIMUS IN PATIENTS WITH RECURRENT CHRONIC HCV INFECTION AFTER ORTHOTOPIC LIVER TRANSPLANTATION: THE SATURN STUDY

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Background and Aims: SATURN (TMC435HPC3016) is an ongoing Phase II open-label study investigating the combination of simeprevir (SMV), daclatasvir (DCV) and ribavirin (RBV) in patients with recurrent chronic hepatitis C virus (HCV) genotype (GT)1b infection after orthotopic liver transplantation. Data from a planned interim 4-week pharmacokinetic (PK)/safety analysis are reported.
Methods: Patients with recurrent chronic HCV GT1b infection (METAVIR score F1–F2) received SMV (150 mg once-daily [QD]), DCV (60 mg QD), and RBV (1–1.2 g/day) and concomitant stable immunosuppressive therapy with cyclosporine A (CsA) or tacrolimus (TAC). Serial PK SMV sampling followed by noncompartmental analysis occurred on day (D)14. PK data were compared with historic noncompartmental Phase II data and Phase III population PK data. Final data from 35 patients, including those with METAVIR score F3–F4, will be available at the time of presentation.
Results: Twenty patients were included in this analysis: CsA, n = 9 (METAVIR F1 n = 4, F2 n = 5); TAC, n = 11 (METAVIR F1 n = 6, F2 n = 5). PK data for SMV on D14 and comparisons with Phase II studies are presented in the Table. When coadministered with CsA, SMV exposure was highly variable and the least squares mean (LSM) ratio was 4.74 for C_{max} and 5.81 for area under the curve (AUC) compared with historic data (SMV without CsA). Although there were no significant safety findings, the SMV dose was reduced to 150 mg every other day in 7/9 patients with high SMV exposures receiving concomitant CsA based on individual patient SMV exposures. On D14 post-dose adjustment, plasma exposures of SMV in those patients were within the range of exposures observed in Phase III studies. When SMV was coadministered with TAC, LSM ratios for C_{max} and AUC were 79% and 85% higher, respectively, than historic data (SMV without TAC) and no SMV dose adjustment was required. Coadministration with SMV did not affect CsA and TAC PK. Grade 3 adverse events (AEs) occurred in 2 patients receiving CsA (hyperbilirubinemia, n = 1; anaemia, n = 1) and 1 patient receiving TAC (pneumonia, hyperbilirubinemia, anaemia). No grade 4 AEs occurred.
Conclusions: In patients with recurrent chronic HCV infection after orthotopic liver transplantation, concomitant use of SMV with CsA resulted in significantly increased plasma SMV concentrations. TAC did not result in a clinically significant increase of SMV exposure. No new safety signals for SMV were identified.
Funded by Janssen R&D, Ireland.

SMV PK parameter	LSM		LSM ratio	90% CI
	TMC435-C205 and TMC435-C206 Reference	TMC435HPC3016 Test		
CsA group				
C_{max}, ng/L	3235 (n = 49)	15321 (n = 9)	4.74	3.12–7.18
AUC_{24h}, ng·h/mL	45202 (n = 48)	262618 (n = 9)	5.81	3.56–9.48
TAC group				
C_{max}, ng/L	3235 (n = 49)	5780 (n = 11)	1.79	1.22–2.62
AUC_{24h}, ng·h/mL	45202 (n = 48)	83808 (n = 11)	1.85	1.18–2.91

CI, confidence interval.

P0835

GERMAN MULTICENTER COHORT ON SOFOSBUVIR-BASED TREATMENTS IN HCV MONO- AND HIV/HCV CO-INFECTED PATIENTS (GECOSO)

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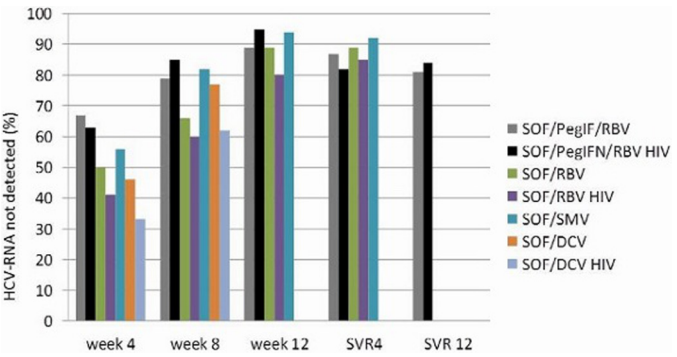
Background and Aims: Sofosbuvir (SOF) and later on simeprevir (SMV) and daclatasvir (DCV) were approved in Europe in 2014 with limited study data. In particular HIV/HCV co-infection and pre-treated patients were not systematically studied. Here, we present real-life data on sofosbuvir-based HCV treatment from Germany.
Methods: In this ongoing multicenter cohort, all patients receiving the following treatment regimens were documented: SOF/ribavirin (RBV), SOF/DCV, SOF/SMV, and SOF/pegylated Interferon (PegIFN)/RBV. For the current analysis due to the limited observational period SVR12 data are pending for all regimens with the exception of SOF/PegIFN/RBV. In April 2015 SVR 12 results for all regimens will be available.
Results: Overall, 395 patients have been enrolled so far. Of those, 288 were HCV-mono-infected and 107 HIV/HCV-co-infected. The HCV genotype (GT) pattern was: GT1 n = 254 (64%), GT2 n = 24 (6%), GT3 n = 85 (21%), GT4 n = 29 (7%). Liver cirrhosis was present in 152/395 (38%) patients. 202/395 (50%) patients were pre-treated with interferon based therapy. Patients were treated as follows (HCV mono-infection/HIV/HCV co-infection): SOF/PegIFN/RBV n = 117/n = 52, SOF/RBV n = 55/n = 19, SOF/DCV n = 87/n = 31, SOF/SMV n = 28/n = 5. The on treatment viral response did not differ substantially between HIV/HCV co-infection and HCV-mono-infection (figure 1). However the preliminary analysis of the data indicates a difference in the on treatment viral kinetics depending on the regimen (figure 1). SOF/PegIFN/RBV showed an SVR12 of 84% in HCV mono-infection compared to 81% in HIV/HCV co-infection. So far <5% of patients discontinued therapy prematurely or were lost to follow up.



Conclusions: In this preliminary analysis, HIV/HCV co-infected and HCVmono-infected patients treated with SOF based therapies showed similar on treatment viral kinetics with different SOF based regimens. For SOF/PegIFN/RBV the SVR12 rate showed no difference between HIV/HCV-coinfected and HCV-monoinfected individuals. However SVR12 seems to be lower in GECOSO than in the NEUTRINO study despite a low discontinuation rate. The lower SVR rate may be attributable to the cohort population containing more difficult-to-treat patients as reported from the HCV-TARGET cohort.

P0836

TREATMENT RESPONSE RATES IN A REAL LIFE COHORT OF PATIENTS WITH ADVANCED LIVER DISEASE AND ORGAN TRANSPLANTATION

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Background and Aims: Interferon free treatments (i-free) containing regimes of Simeprevir (SIM), Sofosbuvir (SOF) and Daclatasvir (DCV) +/− Ribavirin are reimbursed in patients with advanced liver disease (F3–F4) and in post transplantation setting due to Hepatitis C in Austria. Although real life data are emerging from the US, real life data for Europe are still lacking. Therefore we analysed our HCV cohort treated with i-free therapy.
Methods: 94 patients applied to treatment, 9 cases approval refused, 8 waiting for approval, 1 died, 17 approval passed and waiting for start of therapy and 59 started so far. Viral load was measured using COBAS TaqMan HCV quantitative assay, Version 2 (LLOQ: 15 IU/ml) at weeks 1, 2, 3, 4, 8, 12 (24) and follow up 4 and 12. All patients are treated for 12 weeks n = 38 (64.4%) or 24 weeks n = 19 (32.2%) with a combination of SOV+SIM n = 37 (62.7%), SOV+DCV n = 13 (22%), SOV+RBV n = 6 (10.2%) or SOV n = 3 (5.1%).
Results: 59 patients, 42 (71.2%) male, 17 (28.8%) female, median age 57 (IQR: 53–63 years). Distribution of genotype: 1a n = 16 (27.1%), 1b n = 34 (57.7%), 2 n = 1 (1.7%), 3 n = 6 (10.2%) and 4 n = 2 (3.4%). 43 patients have cirrhosis (73%). 35 (81.4%) are compensated, 8 (18.6%) are decompensated. CPS A n = 32 (74.4%), CPS B n = 7 (16.3%), CPS C n = 2 (4.6%). 14 patients (23.7%) are treatment naive, 27 (45.8%) are PegINF based therapy failures and 18 (30.5%) are PI failures. 6 patients (10.2%) are IL28B polymorphism CC, 38 patients (64.4%) are non-CC, 25.8% missing.
HCV PCR results are shown in Table 1.
In our cohort on treatment response at week 4 is not different in patients with a platelet count $<100\times10^9$ or $>100\times10^9$ (p = 0.2). There is also no difference between patients with or without cirrhosis (p = 0.092), IL28B polymorphism CC vs. Non-CC (p = 0.36), compensated and decompensated patients (p = 0.58) and type of prior therapy PegINF based therapy failures vs. PI failures (p = 0.52).
24 out of 59 patients finished therapy, all of them reached an EOT SVR.
10 patients who underwent therapy had prior organ transplantation: OLTX n = 7, NTX n = 2, OLTX+TX n = 1. 7 patients reached EOT SVR, 3 patients are still under therapy, one of them is already HCV PCR negative.
No severe adverse events occurred under therapy. 6 patients (10.1%) got a mild sunburn during outdoor activity.
Conclusions: EOT SVR rates and FUP SVR rates are outstanding independent of patients' baseline characteristics and severity of disease. At EASL we will be able to present data of approximately 120 patients under i-free therapy.

Time of therapy	n	Pos. HCV-PCR	Neg. HCV-PCR
Week 1	48	47	1
Week 2	46	38	8
Week 3	45	30	15
Week 4	44	14	30
Week 8	39	2	37
Week 12	32	0	32
Week 24	6	0	6
Follow up, 4	6	0	6

P0837

EFFICACY OF SOFOSBUVIR AND DACLATASVIR IN HIV/HCV CO-INFECTED PATIENTS WITH ADVANCED FIBROSIS

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Background and Aims: The aim of this study was to assess efficacy and safety of sofosbuvir (SOF) and daclatasvir (DCV) in HIV/HCV coinfected with advanced fibrosis.
Methods: Nineteen patients (3 naive and 16 experienced) with advanced fibrosis received SOF and DCV with or without ribavirin (RVB) (12 and 7 patients respectively) for 24 weeks.
Results: All patients had F4 fibrosis (Fibroscan >12.5 kPa or histological evidence) except one who had F3 fibrosis. Eight (42%) patients had signs of portal hypertension (oesophageal varices and/or ascites). Three patients were listed for liver transplantation. At week 0, the average ALT, bilirubin, platelet count, HCV viral load, fibrosis, MELD score and CD4 count were respectively: 82 IU/L [14–229], 38 μmol/L, 96,000 cells/μL, 5.9 log IU/mL [2.3–7.1], 32 kPa [12–68], 10 [6–15] and 575 cells/mm^3 [204–1618]. Hepatitis C genotype was G1, G3 and G4 in 9, 4 and 6 patients respectively. HIV viral load was lower than 20 cp/mL in all patients. To avoid drug–drug interaction with DCV, cART was changed in 3 (16%) patients or DCV doses adjusted in 6 patients when required. Concerning tolerance, 5 (42%) patients treated with ribavirin developed anaemia, requiring RBV reduction (n = 5) and erythropoietin (n = 2). Four (21%) patients developed infection (urinary tract infection n = 1, herpes n = 1, bronchitis n = 1 and severe pneumonia n = 1). At week 4, HCV viral load was lower than 15 IU/mL in 14 (74%) patients, and it was lower than 15 IU/mL in all patients (100%) at week 8 and week 12. To date, 11 (59%) patients have stopped treatment and all of them have an undetectable HCV viral load (lower than 15 IU/mL). Two listed patients discontinued anti-HCV therapy at week 7 and week 20 and maintained sustained virological response 12 weeks after liver transplantation. Complete results of safety and efficacy with sustained virological response at week 4 and week 12 will be presented.
Conclusions: The association of sofosbuvir and daclatasvir is very effective and safe in HIV/HCV coinfected patients with advanced fibrosis.

P0838

PROGRESSION TO ADVANCED LIVER FIBROSIS IN HIV/HCV-COINFECTED PATIENTS AND PRIORITIZATION OF NEW HEPATITIS C THERAPIES

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Background and Aims: Because of their high cost, the use of direct-acting antivirals (DAA) is being restricted by many governments to chronic HCV-infected individuals with advanced liver fibrosis. However, response rates are lower and toxicities more frequent in this subset of patients.
Methods: All HCV/HIV-coinfected patients followed for at least 3 years at one reference clinic were identified. Liver fibrosis progression (LFP) was defined as a shift from Metavir F0–F2 to F3–F4 estimates (>9.5 kPa) using elastometry.

Results: A total of 527 HIV/HCV-coinfected patients were identified, of whom 344 had F0–F2 at baseline. Peginterferon-ribavirin therapy was given to 205 patients with null-mild fibrosis, of whom 92 (44.9%) achieved sustained virological response (SVR). After a mean follow-up of 53 months, LFP occurred in 5.4% SVR, 25.7% non-SVR and 18% untreated patients (p = 0.005). In multivariate analysis, only achievement of SVR prevented LFP (adjusted hazard ratio 2.1; 95% confidence interval 1.1–4.1; p = 0.01).
In 139 untreated patients, only greater baseline elastometry values predicted LFP in multivariate analysis (adjusted hazard ratio 1.84; 95% CI: 1.03–3.3; p = 0.03). The area under the receiver operating characteristic (AUROC) curve was 79%. A discriminant threshold of 7.1 kPa gave 68% sensitivity and 82% specificity.
Conclusions: In the absence of successful treatment, more than 20% of HIV/HCV-coinfected patients with null-mild liver fibrosis progress to advanced fibrosis within 5 years. Patients with >7.1 kPa (Metavir F2) display the highest risk. Therefore, all coinfected patients with any significant liver fibrosis should be considered as candidates for new DAA-based therapies.

P0839
EVALUATION OF DRUG–DRUG INTERACTIONS BETWEEN THE FIXED-DOSE COMBINATION OF DACLATASVIR/ASUNAPREVIR/BECLABUVIR AND METHADONE OR BUPRENORPHINE/NALOXONE

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Background and Aims: Daclatasvir (DCV; NS5A inhibitor), asunaprevir (ASV; NS3 inhibitor) and beclabuvir (BCV [BMS-791325]; nonnucleoside NS5B inhibitor) are in phase 3 evaluation for hepatitis C virus (HCV) infection as a twice-daily (BID) fixed-dose combination (DCV/ASV/BCV, 30/200/75 mg). HCV is common among injection drug users, and often medically managed with the opioids methadone (MET), or buprenorphine (BUP) + naloxone (NAL) The effect of DCV/ASV/BCV on the pharmacokinetics (PK) of MET or BUP/NAL was assessed.
Methods: An open-label study of the effect of steady-state DCV/ASV/BCV BID on the PK of MET (Part 1; N = 16) or BUP/NAL (Part 2; N = 16) was conducted in HCV-uninfected subjects on stable opioid maintenance. Subjects received daily oral MET (40–120 mg) or BUP/NAL (8/2–24/6 mg), plus DCV/ASV/BCV BID on Days 2–12 plus additional BCV (75 mg BID) to adjust for higher systemic BCV exposure in HCV patients. Serial PK sampling was on Days 1 (opioid alone) and 12. Dose-normalized PK parameters for *R*-, *S*-, and total-MET, BUP and its metabolite norBUP were derived. Geometric mean ratios (GMR; Day12:Day 1) and 90% confidence intervals (90%CI) for C_{max} and AUC_{tau} of each analyte were derived from linear mixed-effect models. No clinically relevant interaction was to be inferred if C_{max} and AUC_{tau} 90%CI were within literature-derived boundaries (R-MET: 0.7–1.43; BUP/norBUP: 0.5–2.0). Safety and opioid pharmacodynamics (PD, withdrawal scales and overdose assessment) were assessed.
Results: Opioid PK parameters are shown. Steady-state DCV/ASV/BCV (+ BCV) BID resulted in a ~30% reduction in dose-normalized R-, S- and total-MET systemic exposures with 90%CI outside prespecified boundaries. However, no loss of MET maintenance efficacy was observed by investigator or PD assessment throughout dosing. No relevant effects were observed on BUP or norBUP PK. All-cause adverse events (AEs, mild/moderate) occurred in: Part 1, 10 subjects (63%); Part 2, 6 subjects (38%). There were no deaths, serious AEs, withdrawal/overdose AEs or discontinuations for AEs. All AEs resolved without sequelae.
Conclusions: Steady-state DCV/ASV/BCV (+ BCV 75 mg) BID was generally well tolerated and had no clinically meaningful effect on the PK of BUP/NAL. A reduction in MET systemic exposure was observed but was not associated with loss of clinical maintenance. No a priori dose adjustments appear warranted when DCV/ASV/BCV is administered to HCV patients on MET or BUP/NAL maintenance.

		Adj. Geo. Mean		GMR (90% CI)
		With DCV/ASV/BCV + BCV	W/O DCV/ASV/BCV + BCV	
R-MET[a]	Cmax, ng/mL	57.0	88.1	0.65 (0.53, 0.79)
	AUCtau, ng·h/mL	1055	1440	0.73 (0.63, 0.85)
BUP	Cmax, ng/mL	7.07	9.30	0.76 (0.65, 0.90)
	AUCtau, ng·h/mL	55.55	60.11	0.92 (0.81, 1.05)
norBUP	Cmax, ng/mL	4.03	4.12	0.98 (0.81, 1.18)
	AUCtau, ng·h/mL	55.66	56.66	0.98 (0.83, 1.17)

[a] S-MET and total MET results similar.
Exposures were normalized to lowest dose of MET or BUP.

P0840
SAFETY AND EFFECTIVENESS OF SOFOSBUVIR-BASED REGIMENS FOR THE TREATMENT OF HEPATITIS C GENOTYPE 3 AND 4 INFECTIONS: INTERIM ANALYSIS OF A PROSPECTIVE, OBSERVATIONAL STUDY

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Background and Aims: HCV genotype (GT) 3 and 4 are common in many regions of the world and optimal treatment regimens continue to evolve. The real-world safety and efficacy of available antiviral options in clinical practice has not been reported. The aim of this study is to evaluate the safety and efficacy of sofosbuvir (SOF) containing regimens for the treatment of patients with GT 3 and 4 in HCV-TARGET (HCVT), a multicentre, prospective, observational cohort study.
Methods: Patients who initiated HCV treatment in clinical practice were enrolled and treated according to the regional standards of care at academic (n = 43) and community medical centres (n = 13) in North America (n = 51) and in Europe (n = 5). Information was collected from the medical records and abstracted into a unique centralized data core. Independent data monitors systematically review data entries for completeness and accuracy. Demographic, clinical, adverse events (AEs) and virological data were collected throughout treatment and post-treatment follow-up
Results: Of 202 patients with GT 3 and 47 with GT4, 110 (54%) and 31 (66%) have completed treatment, respectively. Demographic and treatment regimens are shown in the Table. The majority of patients with GT 3 (91%) were treated with SOF/ribavirin (RBV) whereas patients with GT 4 received peginterferon (PEG) plus SOF/RBV (49%), SOF/RBV (25%) or Simeprevir (SMV)/SOF±RBV (26%). 132 (106 GT 3 and 26 GT 4) patients experienced at least one AE, although most were mild. Among patients with GT 3, five discontinued due to AEs (anemia, flu-like symptoms, nausea, vision loss and intolerance) and 4 for lack of efficacy; 1 was lost to follow-up and 1 underwent liver transplant. One patient with GT 4 stopped due to worsening encephalopathy. As of most recent follow-up, 144/162 GT3 and 34/37 GT 4 had HCV RNA below level of quantitation. Among patients for whom post-treatment data is

Table (abstract P0840): Demographics of all HCV genotype 3 and 4 infection patients who started treatment

Variable	HCV genotype 3			HCV genotype 4				
Treatment regimen	SOF/PEG/RBV	SOF/RBV	Total	SOF/PEG/RBV	SOF/RBV	SOF/SMV	SOF/SMV/RBV	Total
Subjects	18	184	202	23	12	6	6	47
Male, n (%)	12 (67)	114 (62)	126 (62)	18 (78)	11 (92)	4 (67)	4 (67)	37 (79)
Mean age (yrs)	51	55	55	54	59	59	57	56
White race, n (%)	14 (78)	147 (80)	161 (80)	10 (44)	6 (50)	2 (33)	3 (50)	21 (45)
Treatment-experienced, n (%)	10 (56)	92 (50)	102 (50)	16 (70)	9 (75)	3 (50)	5 (83)	33 (70)
Cirrhosis, n (%)	9 (50)	94 (51)	103 (51)	7 (30)	6 (50)	3 (50)	2 (33)	18 (38)
Post-Liver transplant, n (%)	1 (6)	18 (10)	19 (9)	0 (0.0)	1 (8)	0 (0.0)	(0.0)	1 (2)

currently available, 22/38 (58%) and 5/5 (100%) of GT3 patients treated with SOF/RBV and SOF/RBV/PEG, respectively, achieved SVR4 and 14/16 (88%) and 4/4 (100%) of GT4 patients treated with SOF/PEG/RBV and SOF/SIMSMV±RBV, respectively, achieved SVR4. Complete efficacy data will be presented

Conclusions: Preliminary safety and efficacy data from HCVT for patients with HCV genotype 3 and 4 infections suggests that sofosbuvir-containing treatment regimens are generally safe and well tolerated across a broad spectrum of patients and clinical practices.

P0841
SUCCESSFUL COMMUNITY BASED TREATMENT OF HEPATITIS C IN PEOPLE WHO INJECT DRUGS

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Background and Aims: Hepatitis C virus (HCV) treatment uptake is <2% in Australia. A new nurse led service was developed linking The Alfred Hospital to community clinics. The service aimed to increase access to care, targeting current and past people who inject drugs (PWID). A hepatology nurse and specialist physicians attended 3 primary care clinics; all offered opiate substitution therapy (OST). Assessment and management was nurse led. We aim to describe the success of this service engaging and treating PWID.

Methods: A retrospective audit of the service was undertaken. All patients referred to the nurse from service inception in 2010 until 1/8/14 were determined by accessing clinic appointment scheduling. Data collected included; patient demographics, drug, alcohol and psychiatric history, results, treatment uptake and outcome and retention in care.

Results: 462 patients were referred to the community hepatitis nurse service – 344 attended. 279 patients had chronic HCV infection, of these; 184 (66%) were male, 208 (75%) had a psychiatric diagnosis, 145 (52%) were on psychotropic medication, 257 (92%) were current or past PWID, 147 (53%) were on OST, and 99 (35%) reported current injecting drug use (IDU). 203 (73%) had a fibroscan. 15 (5%) patients had significant fibrosis (fibroscan score >9.0 kPa, or clinical evidence such as oesophageal varices).

Fifty-five patients (20%) commenced HCV treatment; 47 had pegylated interferon (PEG) based regimens and 8 were enrolled in PEG-free directly acting antiviral (DAA) trials. Of the 55 patients who commenced treatment, 18 (33%) reported current IDU, 32 (58%) were on OST and 35 (64%) were on psychotropic medication. 14 patients are still undergoing treatment or are <3 months post treatment. Of the remaining 41 patients, 28 completed treatment, 6 ceased due to side effects, 6 fulfilled treatment futility criteria and 1 was lost to follow up. Of the 28 patients who completed therapy, 23 (56%) had a sustained virologic response (SVR). Factors associated with treatment uptake included OST and being on psychotropic medication. Of the 256 patients with chronic HCV who have not had an SVR, 95 (37%) remain engaged in care.

Conclusions: This community based program successfully provided care and interferon based treatment to a significant number of PWID, with an SVR rate comparable to patients treated in tertiary institutions. With the advent of DAAs it highlights the benefits of community based HCV services in managing PWID, a group with a high prevalence of HCV infection.

P0842
MALACHITE-I: PHASE 3B TRIAL OF OMBITASVIR/PARITAPREVIR/R AND DASABUVIR +/− RIBAVIRIN OR TELAPREVIR + PEGINTERFERON/RIBAVIRIN IN TREATMENT-NAÏVE ADULTS WITH HCV GENOTYPE 1

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Background and Aims: In phase 3 trials, regimens of co-formulated ombitasvir/paritaprevir/r (paritaprevir [formerly ABT-450] identified by AbbVie and Enanta, co-dosed with ritonavir [r]) and dasabuvir +/− ribavirin (3D±RBV) demonstrated high efficacy rates and low rates of drug discontinuation due to adverse events (AEs) in patients with chronic HCV genotype (GT) 1 infection. Telaprevir (TPV) plus peginterferon (pegIFN)/RBV remains the standard of care for chronic HCV GT1 infection in many regions. However, this regimen is associated with long treatment duration, suboptimal efficacy, and treatment-limiting AEs related to pegIFN/RBV and exacerbated by TPV-associated rash and anemia. MALACHITE-I is the first multicenter trial to directly compare efficacy and safety of an all-oral direct-acting antiviral regimen (3D±RBV) and TPV+pegIFN/RBV in treatment-naive HCV GT1-infected pts without cirrhosis.

Methods: This multicenter, open-label trial included 311 HCV GT1-infected treatment-naive patients without cirrhosis. Patients received 3D+RBV for 12 weeks, 3D for 12 weeks, or TPV+pegIFN/RBV for 12 weeks and pegIFN/RBV for an additional 12–36 weeks (total treatment duration of 24–48 weeks). Patients with GT1a infection

Table 1 (abstract P0842). Baseline characteristics and treatment efficacy

	3D+RBV		3D	TPV+pegIFN/RBV	
	GT1a (N = 69)	GT1b (N = 84)	GT1b (N = 83)	GT1a (N = 34)	GT1b (N = 41)
Male, n (%)	48 (69.6)	38 (45.2)	40 (48.2)	17 (50.0)	17 (41.5)
Mean age (SD)	46.1 (12.3)	46.2 (11.3)	47.1 (11.3)	44.5 (14.1)	45.9 (10.8)
Fibrosis stage [a] ≥F2, n (%)	19 (27.9)	14 (16.7)	23 (27.7)	10 (29.4)	11 (26.8)
SVR4 rate (ITT), n (%)	67 (97.1)*	83 (98.8)*	81 (97.6)*	29 (85.3)	34 (82.9)
SVR12 rate (ITT), n (%)	67 (97.1)	83 (98.8)	81 (97.6)	Will be presented	Will be presented
On-treatment virologic failure, n (%)	2	0	1	2	5
Post-treatment relapse [b], n (%)	0	1	0	0	1
Non-virologic treatment failure [c], n (%)	0	0	1	3	1

*P-value, based on logistic regression, ≤0.05 for the comparison with the respective TPV+pegIFN/RBV arm.
[a] Fibrosis stage was assessed by liver biopsy scores, FibroScan scores, or FibroTest scores. One GT1a-infected patient receiving 3D+RBV had fibrosis stage missing.
[b] Post-treatment relapse shown through post-treatment week 12 for 3D+RBV and 3D arms and through post-treatment week 4 for TPV+pegIFN/RBV arms.
[c] Reasons for non-virologic failure are missing data, loss to follow-up, and discontinuation of study drug.

Table 2 (abstract P0842). Treatment-emergent adverse events

	3D+RBV (N = 153)	3D (N = 83)	TPV+pegIFN/RBV (N = 75)
Any AE, n (%)	114 (74.5)*	42 (50.6)*	72 (96.0)
Serious AE, n (%)	1 (0.7)*	0*	9 (12.0)
AE leading to drug discontinuation, n (%)	1 (0.7)*	0*	6 (8.0)
AE leading to RBV dose modification, n (%)	7 (4.6)*	0 (NA)	36 (48.0)
Common AEs, n (%)			
Anemia	11 (7.2)*	1 (1.2)*	34 (45.3)
Nausea	32 (20.9)*	7 (8.4)*	29 (38.7)
Pruritus	19 (12.4)*	5 (6.0)*	25 (33.3)
Fatigue	21 (13.7)*	4 (4.8)*	22 (29.3)
Headache	41 (26.8)	16 (19.3)	22 (29.3)
Decreased appetite	6 (3.9)*	1 (1.2)*	16 (21.3)
Pyrexia	4 (2.6)*	2 (2.4)*	16 (21.3)
Rash	12 (7.8)*	0*	16 (21.3)

*P-value ≤0.05 for the comparison with the TPV+pegIFN/RBV arm. Fisher's exact test was used for comparison of AE frequency.

were randomized 2:1 to 3D+RBV and TPV+pegIFN/RBV. Patients with GT1b infection were randomized 2:2:1 to 3D+RBV, 3D, and TPV+pegIFN/RBV. The primary endpoint was SVR12. Patients will be followed for 48 weeks post-treatment.

Results: Patient baseline characteristics are in Table 1. For patients receiving 3D+RBV or 3D, SVR4 and SVR12 rates were 97.1–98.8%; 3 patients had on-treatment failure and 1 patient had post-treatment relapse (Table 1). SVR4 rates were 82.9–85.3% for patients receiving TPV+pegIFN/RBV; SVR12 rates for the TPV+pegIFN/RBV arm will be presented. Rates of common AEs (including anemia, nausea, and pruritus), AEs leading to drug discontinuation or RBV dose modification, and serious AEs were significantly lower in patients receiving 3D±RBV compared to patients receiving TPV+pegIFN/RBV (Table 2). The frequency of RBV-associated AEs, such as anemia, was significantly lower in the 3D±RBV arms compared to the TPV+pegIFN/RBV arm.

Conclusions: In this trial, 12 weeks of 3D±RBV resulted in SVR12 rates of 97.1–98.8% while 12 weeks of TPV with 24–48 weeks of pegIFN/RBV resulted in SVR4 rates of 82.9–85.3% in treatment-naive patients with chronic HCV GT1 infection without cirrhosis. 3D±RBV also demonstrated better tolerability, with ≤0.7% of patients experiencing an AE leading to treatment discontinuation.

P0843

ON-TREATMENT VIRAL KINETICS DO NOT PREDICT SVR IN PATIENTS WITH ADVANCED LIVER DISEASE RECEIVING SOFOSBUVIR IN COMBINATION WITH DACLATASVIR OR SIMEPREVIR FOR 12 WEEKS

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Background and Aims: New direct-acting antiviral (DAA) drugs, including sofosbuvir (SOF), daclatasvir (DCV) and simeprevir (SMV), are available for the IFN-free treatment of patients with chronic HCV infection with advanced fibrosis or cirrhosis. We investigated the predictive role of on-treatment viral kinetics on the virological outcome of IFN-free SOF/DCV and SOF/SMV regimens in the real-life setting

Methods: 240 HCV mono-infected patients with advanced fibrosis (Fibroscan 9.6–12.4 kPa, 42%) or compensated cirrhosis (Fibroscan >12.5 kPa, 58%) received SOF/DCV (78%) or SOF/SMV (22%) for 12 weeks without RBV, regardless of their genotype (GT). The majority of patients were males (63%) and treatment-experienced (68%). The GT distribution was GT1b (31%), GT1a (25%), GT4 (24%), GT3 (17%) and other GTs (3%). SVR was available at post-treatment week 4 (SVR4) in 86 patients.

Results: Overall, 94% (81/86) of patients achieved SVR4. Three different patterns of virologic response were observed: (a) Rapid response: HCV RNA <12 IU/mL (detected or not) at week 4, n = 41 (48%); (b) Early response: HCV RNA >12 IU/mL at week 4 and HCV RNA <12 IU/mL (target detected or not) at week 8, n = 23 (27%); (c) Slow response: HCV RNA >12 IU/mL at weeks 4 and 8 and HCV RNA still detectable at week 12, i.e. end-of-treatment (EOT), n = 22 (25%). In the latter group, all but one patient with an HCV RNA=23 IU/mL had a detectable HCV RNA <12 IU/mL. In the

slow responder group, the proportion of treatment-experienced patients and GT4 tended to be higher than in the overall population, 82% (18/22) and 41% (9/22), respectively. The other baseline characteristics were similar, male 55% (12/22), cirrhosis 59% (13/22). As shown in the Table, SVR4 did not vary according to the on-treatment viral kinetics. Strikingly, 20/22 (91%) slow responders who still had detectable HCV RNA at EOT achieved an SVR4.

Conclusions: Based on our results in a cohort of real-life patients with advanced liver disease or cirrhosis, on-treatment viral kinetics do not predict the virological outcome of the new IFN-free SOF-based regimens. Thus, viral kinetics monitoring should not be used to alter the planned treatment regimen. In particular, most patients with detectable low-level HCV RNA at the end of treatment at week 12 achieve an SVR, raising the question as to the mechanisms involved in post-treatment clearance. SVR12 results and multivariate analysis of outcome predictors will be presented for the 240 patients.

	Rapid response N = 41	Early response N = 23	Slow response N = 22
SVR4, n/N (%)	38/41 (93)	23/23 (100)	20/22 (91)

P0844

IS DUAL INTERFERON AND RIBAVIRIN TREATMENT SAFE AND EFFICACIOUS AMONG THE CUPIC COHORT? – A RETROSPECTIVE COHORT STUDY

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Background and Aims: Hepatitis C virus is an RNA virus capable of establishing chronic infection which is associated with a significant risk of developing cirrhosis. The introduction of triple therapy protease inhibitors promised markedly improved sustained viral response rates albeit with a slightly worsened side effect profile. The promise of improved response and the already known poor response to interferon and ribavirin based dual therapy among cirrhotic patients prompted many physicians to defer treatment, a process which became known as warehousing.

The recent publication of the French Early access programme also known as the CUPIC study which targeted patients with advanced liver disease identified worryingly high rates of serious complications and a number of deaths among cirrhotic patients with platelet counts below 100,000 and albumin <35 g/L who were treated with triple therapy. We noted that two of these deaths occurred in patients who had commenced dual therapy but prior to commencing treatment with protease inhibitors.

We hypothesised that dual therapy may have had similar outcomes to triple therapy.

Methods: We performed a multicentre retrospective review of treatment outcomes among cirrhotic patients in the period between 2003 and 2012 in two of the leading centres for HCV treatment in the Republic of Ireland.

We identified 153 cirrhotic patients who had undergone treatment with dual therapy in that period.

Results: Our results are summarised in Table 1.

There were no mortalities among our cohort of patients. Although our cohort was only one third the size of the CUPIC study our discontinuation rate due to either death or adverse drug effects was 9% among patients with both low platelets and albumin levels which contrasts with a discontinuation rate of 44% among the CUPIC cohort. Among genotype 1 patients with low platelets and albumin had a non response rate of 86% and an SVR rate of 14%. SVR rates were markedly lower among patients with both low platelet and albumin levels. Conversely, SVR rates among subjects with preserved platelets counts and albumin levels were comparable to published rates for non cirrhotic patients and this was also true for when analysed by genotype.

Conclusions: Dual therapy has an acceptable safety profile among cirrhotic patients. There appears to be an associated between low platelets and albumin levels and poor lower rates of SVR.

These findings suggest that the adverse outcomes reported in CUPIC may have been the result of protease inhibitor use.

Table 1.

	Platelet count >100,000/mm^3		Platelet count ≤100,000/mm^3	
	Result	n/N (%)	Result	n/N (%)
All genotypes (n = 135)				
Albumin ≥35 g/L	**SVR**	**55/90 (61%)**	**SVR**	**9/23 (39.1%)**
	Relapse	17/90 (19%)	Relapse	7/23 (30.4%)
	IFN non-response	15/90 (17%)	IFN non-response	4/23 (17.4%)
	Side-effect related d/c	3/90 (3%)	Side-effect related d/c	3/23 (13%)
Albumin <35 g/L	**SVR**	**3/5 (60%)**	**SVR**	**1/11 (9%)**
	Relapse	2/5 (40)	Relapse	3/11 (27%)
	IFN non-response	0	IFN non-response	6/11 (55%)
	Side-effect related d/c	0	Side-effect related d/c	1/11 (9%)
Genotype 1 (n = 52)				
Albumin ≥35 g/L	**SVR**	**15/34 (41%)**	**SVR**	**1/6 (17%)**
	Relapse	6/34 (18%)	Relapse	3/6 (50%)
	IFN non-response	12/34 (35%)	IFN non-response	2/6 (33%)
	Side-effect related d/c	2/34 (6%)	Side-effect related d/c	0
Albumin <35 g/L	**SVR**	**2/3 (67%)**	**SVR**	**1/7 (14%)**
	Relapse	1/3 (33%)	Relapse	0
	IFN non-response	0	IFN non-response	6/7 (86%)
	Side-effect related d/c	0	Side-effect related d/c	0
Genotype 2/3 (n = 83)				
Albumin ≥35 g/L	**SVR**	**41/56 (73%)**	**SVR**	**8/17 (47%)**
	Relapse	11/56 (20%)	Relapse	4/17 (23.5%)
	IFN non-response	3/56 (5%)	IFN non-response	2/17 (11.8%)
	Side-effect related d/c	1/56 (2%)	Side-effect related d/c	3/17 (17.6%)
Albumin <35 g/L	**SVR**	**1/2 (50%)**	**SVR**	**1/4 (25%)**
	Relapse	1/2 (50%)	Relapse	2/4 (50%)
	IFN non-response	0	IFN non-response	0
	Side-effect related d/c	0	Side-effect related d/c	1/4 (25%)

P0845

COMPARATIVE STUDY ON THE EFFECTIVENESS OF SIMEPREVIR OR TELAPREVIR IN COMBINATION WITH PEGINTERFERON AND RIBAVIRIN FOR CHRONIC HCV GENOTYPE 1B INFECTION

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Background and Aims: The addition of hepatitis C virus (HCV) NS3/4A protease inhibitors to the peginterferon and ribavirin (triple therapy) has greatly improved treatment outcome, but there has not been adequate study of the difference in effectiveness of the first- and second-generation of NS3/4A protease inhibitors. The aim of this study was to compare the treatment outcome of simeprevir (SMV)- or telaprevir (TVR)-based triple therapy.

Methods: This multicenter cohort study consisted of 835 consecutive Japanese HCV genotype 1b patients treated in a clinical setting, 808 of whom were enrolled (SMV 283 and TVR 525). The

interleukin-28B (IL28B) genotype at rs8099917 was determined for all studied patients and mild fibrosis was determined by METAVIR score F0–2 or FIB-4 index <3.25. Logistic regression was done after propensity score matching to assess the sustained virological response at week 12 after the end of treatment (SVR12).
Results: The SVR12 rates of the patients treated with SMV- or TVR-based triple therapy were 82.3% and 81.1%, respectively, by intention-to-treat analysis. Propensity score matching of the entire study population yielded 275 matched pairs. No significant difference was found between the SVR12 rates of the SMV (81.8%) and TVR (80.0%) groups (P = 0.59). Moreover, no significant difference in the SVR12 rate was found for treatment-naïve patients (SMV: 86.6% and TVR: 84.5%) or those with prior relapse (92.5% and 88.5%), prior partial response (64.0% and 61.5%), or prior null response (30.4% and 41.7%). For treatment-naïve patients, IL28B TT genotype (odds ratio [OR] 6.29; P < 0.0001) and mild fibrosis (OR 2.81; P = 0.0091) were independent pretreatment predictors of SVR12. For patients with prior relapse, the SVR12 rate by per-protocol analysis was extremely high for both SMV (96.3%) and TVR (91.8%) groups. In analysis of difficult-to-treat groups with prior partial/null response, IL28B TT genotype (OR 5.41; P = 0.015) and mild fibrosis (OR 6.34; P = 0.0047) for the prior partial response group and mild fibrosis (OR 6.50; P = 0.0038) for the prior null response group were independent predictors of SVR12, irrespective of the NS3/4A protease inhibitor used.
Conclusions: The treatment outcome and predictors of SVR12 by NS3/4A protease inhibitor-based triple therapy were very similar between groups treated with SMV or TVR for chronic HCV genotype 1b infection. When considering the choice of triple therapy for difficult-to-treat patients, fibrosis status may be the most useful factor.

P0846
EVALUATION OF ADHERENCE TO TELAPREVIR (TVR) AND BOCEPREVIR (BOC) USING DIFFERENT THRESHOLDS

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Background and Aims: Information about optimal adherence to protease inhibitors (PIs) in patients with chronic hepatitis C (CHC) receiving triple therapy is very scarce.
The aim of our study was to analyse the sustained virological response at week 12 (SVR12) according to adherence in patients receiving boceprevir (BOC) or telaprevir (TVR) using different thresholds. Secondary aim was to establish the factors related to non-adherence.
Methods: Patients receiving BOC or TVR between May 2012 and April 2014 were included. We excluded those patients with HIV-coinfection. Demographic, virological and clinical data were analysed. Current outpatient medication was collected and Charlson comorbidity index was calculated. Non-adherent patients were defined as those receiving less than 80% of peg-interferon or ribavirin. For PIs, 80, 85, 90 and 95% adherence thresholds were analysed. Discontinuation of treatment in non-responders was not considered as lack of adherence. We compared the percentage of adherent and non-adherent patients achieving the SVR12 and the factors related to a low adherence.
Results: Fifty-seven patients were included: 44 (77.2%) received TVR. The percentage of adherent patients for each threshold was: 73.7% (n = 42) for 80/80/80 or 80/80/85 or 80/80/90 and 71.9% (n = 41) for 80/80/95. The rate of SVR12 in adherent vs. non-adherent patients was: 69% vs. 40% for 80/80/80 or 80/80/85 or 80/80/90 (p = 0.066 for the three thresholds) and 70.7% vs. 37.5% (p = 0.033) for 80/80/95. Advanced fibrosis stage (F3–F4) (p = 0.030), a Charlson index ≥2 (p = 0.003) and the number of concomitant drugs (p = 0.085) were related to low (<80/80/95) adherence. Only the Charlson index ≥2 (OR = 0.120: 95% CI: 0.029–0.491) was independently associated with a low adherence (<80/80/95).
Conclusions: An adherence threshold of 95% for BOC and TVR had the best predictive value for SVR12. Moreover, one third of the patients treated with TVR or BOC were non-adherent due to the presence of comorbidities and advanced disease.

P0847
MALACHITE-II: PHASE 3B TRIAL OF OMBITASVIR/ PARITAPREVIR/R AND DASABUVIR + RIBAVIRIN OR TELAPREVIR + PEGINTERFERON/RIBAVIRIN IN PEGINTERFERON/RIBAVIRIN TREATMENT-EXPERIENCED ADULTS WITH HCV GENOTYPE 1

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Background and Aims: Telaprevir (TPV) plus peginterferon (pegIFN)/ribavirin (RBV) remains a standard therapy for chronic HCV genotype (GT) 1 infection in many regions although its efficacy is suboptimal in treatment-experienced patients (pts), particularly prior null responders. In addition, pegIFN/RBV toxicity is often treatment-limiting and TPV is associated with rash and increased risk of anemia. In phase 3 trials, IFN-free regimens of co-formulated ombitasvir/paritaprevir/r (paritaprevir [formerly ABT-450] identified by AbbVie and Enanta, co-dosed with ritonavir [r]) and dasabuvir+ribavirin (3D+RBV) demonstrated high efficacy rates and low rates of drug discontinuation due to adverse events (AEs) in pegIFN/RBV-experienced pts with chronic HCV GT1 infection. MALACHITE-II is the first multicenter trial to directly compare efficacy and safety of an all-oral direct-acting antiviral regimen (3D+RBV) and TPV+pegIFN/RBV in pegIFN/RBV-experienced HCV GT1-infected pts without cirrhosis.
Methods: This open-label trial included 148 HCV GT1-infected prior pegIFN/RBV relapsers, partial responders, and null responders without cirrhosis. Pts received 3D+RBV for 12 weeks or TPV+pegIFN/RBV for 12 weeks and pegIFN/RBV for an additional 12–36 weeks (total treatment duration 24–48 weeks). Pts were randomized 2:1 to 3D+RBV or TPV+pegIFN/RBV. Primary endpoint was SVR12. Pts will be followed for 48 weeks post-treatment.
Results: Pts were stratified by HCV subtype (1a, 1b) and prior pegIFN/RBV response. Demographic/baseline characteristics were balanced between arms. SVR4 and SVR12 rate was 99.0% for 3D+RBV (Table). SVR4 rate was 68.1% for TPV+pegIFN/RBV; SVR12 rate for TPV+pegIFN/RBV will be presented. In each arm, nearly half the pts were prior null responders to previous pegIFN/RBV treatment. In prior null responders, SVR4/SVR12 rate was 100% (49/49) for 3D+RBV and SVR4 rate was 56.5% (13/23) for TPV+pegIFN/RBV. Rates of any AE, serious AE, AE leading to discontinuation, and AE leading to RBV dose modification were significantly lower for 3D+RBV compared to TPV+pegIFN/RBV (Table). Frequency and severity of RBV-associated AEs, such as anemia, were significantly lower for 3D+RBV compared to TPV+pegIFN/RBV.

Conclusions: In this trial, 12 weeks of 3D+RBV resulted in an SVR12 rate of 99.0% and TPV+pegIFN/RBV resulted in an SVR4 rate of 68.1% in pegIFN/RBV-experienced pts with chronic HCV GT1 infection without cirrhosis. 3D+RBV also demonstrated better tolerability, with no pt experiencing AEs leading to treatment discontinuation.

Table: Baseline characteristics, efficacy and treatment-emergent adverse events

	3D+RBV (N = 101)	TPV+pegIFN/RBV (N = 47)
Male, n (%)	55 (54.5)	28 (59.6)
Mean age (SD)	46.9 (12.2)	45.0 (10.4)
HCV GT1a, n (%)	19 (18.8)	7 (14.9)
Fibrosis stage [a] ≥F2, n (%)	22 (21.8)	15 (31.9)
SVR4 rate (ITT)		
All patients, n/N (%)	100/101 (99.0)*	32/47 (68.1)
Prior null responders, n/N (%)	49/49 (100)*	13/23 (56.5)
SVR12 rate (ITT)		
All patients, n/N (%)	100/101 (99.0)	Will be presented
Prior null responders, n/N (%)	49/49 (100)	Will be presented
On-treatment virologic failure, n	0	9
Post-treatment relapse [b], n	0	2
Non-virologic treatment failure [c], n	1	4
Any AE, n (%)	63 (62.4)*	43 (91.5)
Serious AE, n (%)	1 (1.0)*	5 (10.6)
AE leading to drug discontinuation, n (%)	0*	5 (10.6)
AE leading to RBV dose modification, n (%)	2 (2.0)*	15 (31.9)
Common AEs, n (%)		
Headache	29 (28.7)	21 (44.7)
Nausea	10 (9.9)*	20 (42.6)
Pruritus	13 (12.9)*	19 (40.4)
Asthenia	8 (7.9)*	16 (34.0)
Anemia	3 (3.0)*	15 (31.9)
Pyrexia	2 (2.0)*	14 (29.8)
Neutropenia	1 (1.0)*	13 (27.7)
Anal pruritus	0*	12 (25.5)
Cough	7 (6.9)*	12 (25.5)
Fatigue	12 (11.9)	12 (25.5)
Rash	3 (3.0)*	12 (25.5)

*P-value ≤0.05 for the comparison with the TPV+pegIFN/RBV arm. For SVR4, P-value is based on logistic regression. Fisher's exact test was used for comparison of AE frequency.

[a] Fibrosis stage was assessed by liver biopsy scores, FibroScan scores, or FibroTest scores.

[b] Post-treatment relapse shown through post-treatment week 12 for 3D+RBV arm and through post-treatment week 4 for TPV+pegIFN/RBV arm.

[c] Non-virologic treatment failure includes premature drug discontinuation and missing SVR data.

P0848

EFFICACY OF RESPONSE-GUIDED PEGYLATED INTERFERON AND RIBAVIRIN THERAPY FOR PEOPLE WHO INJECT DRUGS WITH HCV GENOTYPE 2/3 INFECTION: THE ACTIVATE STUDY

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Background and Aims: Despite HCV guidelines advocating for treatment among people who inject drugs (PWID), treatment uptake remains low. The aim of this study was to evaluate response-guided PEG-IFN alfa-2b/RBV treatment for chronic HCV genotypes 2/3 infection among PWID.

Methods: ACTIVATE is a phase IV open-label, multicentre, international trial. Participants with chronic HCV genotype 2/3 and active (previous 24 weeks) injecting drug use or receiving opioid substitution treatment (OST) were recruited between 2012 and 2014. Participants received directly observed PEG-IFN (1.5 μg/kg/week) and self-administered RBV (800–1400 mg daily, weight-based). Participants with a rapid virological response (RVR) received 12 weeks (shortened duration) and those without RVR received 24 weeks (standard duration) therapy. The primary efficacy endpoint was SVR at 12 weeks (SVR12) by ITT analysis. A preliminary ITT analysis included only patients having completed follow-up to SVR12 or having discontinued therapy prior to that time-point.

Results: Overall, 93 participants initiated treatment (mean age 42 years; 82% men; 87% HCV genotype 3). At baseline, 35% (n = 33) were receiving OST [15 (16%) with injecting in the previous 12 weeks], 43% (n = 40) had injected in the past 4–12 weeks and 22% (n = 20) had injected in the last month. In follow up, 66% (n = 61) achieved RVR (shortened treatment), 28% (n = 26) did not (standard treatment), and 6% (n = 6) discontinued therapy prior to week 4. In the preliminary ITT population (n = 75), the ETR and SVR were 83% (n = 62) and 59% (n = 44), respectively. SVR was 69% (38 of 55) in those with an RVR (shortened treatment) compared to 43% (6 of 14) in those without an RVR (standard treatment). SVR was similar among OST (52%, n = 12), non-OST injecting in past 4–12 weeks (79%, n = 11) and non-OST injecting in past month (55%, n = 21, $P = 0.238$) sub-groups. In multivariate analyses, after adjusting for age, gender, HCV genotype, baseline HCV RNA level and baseline injecting risk behaviour (OST vs. non-OST), RVR was the only factor associated with SVR (adjusted OR = 4.38; 95% CI: 1.05, 18.31; $P = 0.043$).

Conclusions: Among PWID with chronic HCV genotypes 2/3, on-treatment RVR was a strong predictor of SVR in patients receiving a 12 week course of PEG-IFN/RBV combination therapy, even in patients actively injecting drugs when treatment was initiated. These data provide further support for recommendations advocating HCV treatment in active PWID and the use of innovative shorter therapies in this context.

P0849

REAL-WORLD EFFECTIVENESS OF SOFOSBUVIR (SOF), TELAPREVIR, AND BOCEPREVIR (T, B) BASED THERAPY FOR HEPATITIS C VIRUS (HCV): AN ANALYSIS IN A LARGE INTEGRATED HEALTH CARE SYSTEM

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Background and Aims: New direct-acting antiviral (DAA) agents have become an integral component of treatment for HCV infection but little is known about their real-world effectiveness particularly in community practice. To optimize therapy, Kaiser Permanente Northern California (KPNC) established an HCV care team at each of 21 medical centers, which included a Gastroenterologist or Infectious Disease specialist, Clinical Pharmacist, Nurse and Hepatologist at each medical center. We compared virologic responses of patients receiving DAA-based therapy to those reported in clinical trials, including FDA approval documents.
Methods: Approval was obtained from the KPNC Institutional Review Board. HCV patients who completed T or B by 30AUG13 or SOF [with ribavirin (RBV) for genotype (GT) 2 or PEG-interferon (PEG-IFN)/RBV for non-GT2] based therapy by 30SEP14 were identified and analyzed on an intent-to-treat basis. Sustained viral response 12 (SVR12) was defined as undetectable (UD) HCV RNA 12 weeks after completing therapy.
Results: For GT1 treatment naïve (TN) patients treated with SOF/PEG-IFN/RBV, we found an SVR12 of 73.3%. For GT2 TN with SOF/RBV, we found the SVR12 was 92.6%. For GT1 with T/PEG-IFN/RBV, we found SVR12's of 65% and 59%, for TN and treatment experienced (TE), respectively, and for GT1 with B/PEG-IFN/RBV, the SVR12's were 56% and 61%, for TN and TE, respectively. Detailed results and comparisons to published clinical trials are located in Figure 1.
Conclusions: Our purely community-based results were similar to those observed in randomized controlled trials and included comparisons of the more difficult to tolerate interferon-based regimens. We believe our multidisciplinary care team provided a similar framework to the intense support often provided in clinical trials.

DAA	Genotype	Treatment History	Study	SVR 12 %	N	P Value (Fisher's Exact Test)
Sofosbuvir PEG-Interferon Ribavirin	1	Naïve	NEUTRINO	89.4	292	0.77
	1	Naïve	KPNC	73.3	30	
Sofosbuvir Ribavirin	2	Naïve	VALENCE	96.9	32	0.57
	2	Naïve	FISSION	93	73	0.93
	2	Naïve	KPNC	92.6	54	
Telaprevir	1	Naïve	KPNC	65%	222	
			Jacobson, IM, et al, NEJM 2011;364;25	75%	363	0.76
	1	Previously Treated	KPNC	59%	100	
			Zeuzem, S, et al, NEJM 2011;364;25	57%	97	0.65
Boceprevir	1	Naïve	KPNC	56%	287	
			Poordad, F, et al, NEJM 2011;364;13	75%	708	0.76
	1	Previously Treated	KPNC	61%	72	
			Bacon, BR, NEJM 2011;364;13	65%	299	0.80

Figure 1.

P0850

PERCENT OF SUBJECTS EXPERIENCING LIVER MORBIDITY OVER A LIFETIME HORIZON WITH ABBVIE 3D (ABT-450/RITONAVIR/OMBITASVIR AND DASABUVIR) VERSUS NO TREATMENT

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Background and Aims: Treating and curing patients with hepatitis C virus (HCV) is associated with reductions in long-term morbidity. Additionally, earlier HCV treatment is thought to reduce morbidity compared to treatment of HCV in later disease stages. Newer therapies for HCV are available that offer substantially improved sustained virologic response (SVR) rates. It is unclear what the impact these drugs will be on long-term morbidity and mortality. The aim of this study was to compare liver disease morbidity rates for HCV patients in varying disease stages who are treated with AbbVie 3D (ABT-450/ritonavir/ombitasvir and dasabuvir) +/− ribavirin compared to no treatment.
Methods: A Markov health state model estimated lifetime outcomes for patients treated with the AbbVie 3D regimen and no treatment. The model had 13 states including 8 disease progression states (F0–F4, decompensated cirrhosis [DCC], hepatocellular carcinoma [HCC], and liver transplant [LT]), 3 SVR states (based on baseline fibrosis score), and 2 mortality states (liver-related and non-liver-related death). Transition rates were derived from previous models. Population characteristics and efficacy rates were based on the AbbVie 3D phase 3 clinical trials. The model was run over a lifetime horizon. Analyses were run separately for each fibrosis score and for treatment naïve and treatment experienced patients. Rates of DCC, HCC, and LT were analyzed.
Results: Lower rates of DCC, HCC, and LT were seen across all fibrosis stages (F0–F4) in HCV patients treated with AbbVie 3D compared to no treatment (Table 1). Rates were 5 to 7 fold lower in the treated HCV patients compared to untreated patients, with an except in HCC among the F4 patients (e.g., 28.5% versus 28.9% for untreated and treated naïve patients, respectively). Rates of HCC were similar between the treated and untreated F4 patients likely due to the excess risk of HCC that remains for compensated patients despite viral clearance. Morbidity rates were similar among treatment naïve and treatment experienced patients of the same disease stage and rates of morbidity rose with increasing baseline disease severity.
Conclusions: The percent of HCV patients experiencing liver morbidity – DCC, HCC, and LT – was significantly reduced with AbbVie 3D treatment compared to HCV patients who were not treated. Rates of morbidity were higher when treatment was deferred to patients with greater baseline disease severity.

Table 1. Percent of subjects experiencing liver morbidity over a lifetime horizon with AbbVie 3D versus no treatment

Fibrosis stage	Liver morbidity					
	Decompensated cirrhosis		Hepatocellular carcinoma		Liver transplant	
	No treatment	AbbVie 3D	No treatment	AbbVie 3D	No treatment	AbbVie 3D
Treatment naive						
F0 (100%)	18.2%	2.9%	10.0%	1.6%	4.0%	0.6%
F1 (100%)	26.0%	4.4%	14.3%	2.4%	5.9%	0.9%
F2 (100%)	33.4%	6.0%	18.5%	3.3%	7.8%	1.3%
F3 (100%)	41.7%	7.9%	23.1%	4.3%	10.1%	1.8%
F4 (100%)	51.2%	9.7%	28.5%	28.9%	12.8%	6.8%
Treatment experienced						
F0 (100%)	17.6%	2.6%	9.6%	1.4%	3.8%	0.5%
F1 (100%)	25.1%	3.9%	13.8%	2.1%	5.6%	0.8%
F2 (100%)	32.3%	5.3%	17.9%	2.9%	7.5%	1.2%
F3 (100%)	40.4%	7.1%	22.4%	3.9%	7.9%	1.6%
F4 (100%)	49.7%	8.5%	27.7%	27.4%	12.3%	6.3%

P0851

EFFECTIVENESS OF SOFOSBUVIR AND RIBAVIRIN FOR GENOTYPE 2 AND 3 HCV AMONG NON-CIRRHOTIC, CIRRHOTIC, AND POST-TRANSPLANT PATIENTS AT 3 US MEDICAL CENTERS

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Background and Aims: Sofosbuvir (SOF) and Ribavirin (RBV) are effective for Genotype 2 and 3 Hepatitis C (HCV) but randomized controlled trials demonstrated variability in the response rates for this group, especially those with cirrhosis and prior treatment. The aim of this work is to evaluate the virological response and safety of SOF+RBV in treating G2 and G3 HCV including cirrhotics, liver transplant (LT) wait-list and LT recipients.

Methods: This multicenter effort analyzed data from 3 academic medical centers in the United States. Relevant demographic, clinical, and virologic data was evaluated in patients who have completed or continue on HCV treatment.

Results: At present, 94 patients (56% male, 7% non-white, 52% genotype 3, 48% genotype 2, 48% with cirrhosis, 14% decompensated) have been treated with SOF+RBV, approximately 70% have completed treatment. The mean MELD and CTP scores were 9 (range 6–18) and 6 (range 5–10), respectively. 21% (10/46) of the cirrhotic patients were awaiting LT and 17 (18%) were post LT. In total, 40% previously failed or did not tolerate peginterferon-based treatments. All patients had estimated GFR >30 mL/min. To date, 97% (56/58) patients completing treatment achieved an end of treatment response (EOTR). Two patients (3%) relapsed immediately after treatment, both cirrhotic. Twenty-six additional patients continue on treatment with 82% achieving treatement week 4 HCV negativity or were below the lower limit of quantification. One pre-transplant cirrhotic stopped SOF+RBV at week 8 due to viral breakthrough. Five other cirrhotics did not complete treatment (1 variceal bleed, 1 persistent nausea/vomiting, 1 hyperbilirubinemia post TIPS, 2 non-compliance). Among the decompensated cirrhotics, 4 patients developed complications (1 SBP, 1 variceal bleed, 1 hyperbilirubinemia post TIPS, 1 overt HE), and 1 compensated cirrhotic developed ascites. One patient who was able to stop diuretics and another was able to reduce diuretic doses. There was not a significant change in mean MELD (9 vs 10) or CTP (5.6 vs 5.6) from drug initiation to end of treatment in cirrhotics.

Conclusions: SOF+RBV was effective for this G2 and G3 HCV population enriched with difficult to treat patients including cirrhotics and post-LT patients. Significant improvement in ascites and/or hepatic encephalopathy was not observed in this population. SVR12 data will be reported when available.

P0852

CLINICAL CHARACTERISTICS AND OUTCOMES OF CHRONIC HEPATITIS C (CHC) PATIENTS TREATED WITH NEWER DIRECT-ACTING ANTIVIRAL (DAA)-BASED REGIMENS FROM A LARGE US PAYER PERSPECTIVE

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Background and Aims: To describe, from a large US payer-perspective, current clinical characteristics, health care utilization and cost per sustained virologic response (SVR) of CHC patients treated with newer DAA-based regimens using claims-linked lab data.

Methods: The Optum Research Database (ORD) with medical and pharmacy claims-linked lab data for commercially-insured patients and Medicare Advantage beneficiaries was used. Patients with CHC aged ≥18 years treated with simeprevir (SMV) and/or sofosbuvir (SOF) regimens after Nov2013 and with 6-months baseline health plan enrollment were followed from treatment initiation until Jun2014. ORD contained 123.1M cumulative lives as of Jun2014.

Results: 1,844 CHC patients were treated with SOF+RBV (33%) SOF +PEG+RBV (32%), SMV+SOF (29%) and SMV+SOF+RBV (6%) (n = 51 were treated with other regimens and excluded from this analysis). Less than half of patients had a diagnosis of cirrhosis (37%). 24% had claims-based evidence of end-stage liver disease. Over half (65%) were male; 68% white and 14% black; and mean age was 57.5 years (SD=9.5 years). One-third (33%) of patients were Medicare beneficiaries and 67% were commercially insured.

Table: Baseline liver disease severity by treatment regimen

Liver disease severity	Total (N = 1,844)		SOF + RBV (N = 615)		SOF + RBV + PEG (N = 581)		SMV + SOF (N = 534)		SMV + SOF + RBV (N = 114)		p-value
	n	%	n	%	n	%	n	%	n	%	
Claims-based evidence of cirrhosis or ESLD	778	42.20	238	38.70	180	30.98	286	53.56	74	64.91	<0.001
Baseline APRI [a,b]											
0 to <0.5	225	34.19	68	31.19	94	43.72	50	27.03	13	32.50	0.003
0.5–1.5	240	36.47	82	37.61	76	35.35	69	37.30	13	32.50	0.904
>1.5	193	29.33	68	31.19	45	20.93	66	35.68	14	35.00	0.008
Baseline FIB-4 [b,c]											
0 to <1.45	196	29.79	65	29.82	81	37.67	36	19.46	14	35.00	<0.001
1.45–3.25	242	36.78	74	33.94	88	40.93	73	39.46	7	17.50	0.009
>3.25	220	33.43	79	36.24	46	21.40	76	41.08	19	47.50	<0.001
No valid APRI or FIB-4 value	1,186	64.32	397	64.55	366	62.99	349	65.36	74	64.91	0.867

[a] APRI = (AST / normality upper limit) / platelet [10^9/L] ×100. AST and platelet values must occur within 30 days of each other.

[b] FIB-4 = (age [years] × AST [IU/L]) / (platelets [10^9/L] × sqrt(ALT [IU/L]). AST, platelet and ALT values must occur within 30 days of each other.

[c] When percentages are presented for valid values for APRI or FIB-4, patients without available values for the result in the denominator are not included.

Mean treatment duration among those with completed regimens (n = 339) was 10.7 weeks; over half of regimens had treatment durations of >11 to ≤15 weeks (57%). Most common prescribing provider type was gastroenterology (54%). Among patients with genotype (GT) results available (n = 597), 1a (51%) was most common, followed by 1b (18%) and 2 (17%). Percentage of patients by GT with SOF+PEG+RBV, SOF+RBV and SMV+SOF±RBV regimens were: GT1a – 44%, 7% and 49%, respectively; GT1b – 41%, 10%, 49%; GT2 – 1%, 99%, 0%; GT4 – 94%, 6%, 0%. Approximately one-third of patients with liver function test results (n = 658) had baseline advanced liver fibrosis (29% with APRI >1.5; 33% with FIB-4 >3.25). Significant variations in treatment regimen were seen across liver disease severity subgroups, including those with advanced liver fibrosis most often being treated with SMV+SOF±RBV (see table). Health care resource utilization and cost per SVR will be examined when post-treatment outcomes data are available.

Conclusions: The analysis provides current CHC treatment patterns in a large US payer database, evidence of potential variation in newer DAA treatment utilization by clinical characteristics, and suggests that a significantly higher proportion of patients receiving SMV+SOF±RBV had advanced liver fibrosis.

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P0853

SERUM CYSTATIN C AND NGAL LEVELS AT THE OUTSET OF PROTEASE INHIBITOR ANTIVIRAL THERAPY POSITIVELY PREDICT RENAL DYSFUNCTION DEVELOPMENT AND ANAEMIA SEVERITY

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Background and Aims: The PAN study reported renal dysfunction in 4.7% and 6.6% of patients on Hepatitis C (HCV) antiviral protease inhibitors (PI) therapy. Risk factors associated with eGFR decline to <60 mL/min at treatment week 12 (TW12) were diabetes and arterial hypertension. Additionally, this eGFR was associated with lower TW12 haemoglobin (Hb) levels.

Serum NGAL is a biomarker for renal tubular cell injury and red blood cell production suppressant, while Cystatin C is a biomarker of glomerular function. Thus could NGAL and Cystatin C predict renal function decline and anaemia severity in cirrhotic and non-cirrhotic patients on PI therapy.

Methods: 33 patients with HCV genotype 1 undergoing PI (bocepravir or telaprevir) based therapy had serum NGAL and Cystatin C measurements at the following time points: treatment initiaton (TW0), TW12, end of treatment (EOT) (median 41 weeks) and 24 weeks post treatment (FUW24), using commercially available Quantikine ELISA kits from R&D systems. Absorbance measured at 450 nm on a microplate reader. Renin, creatinine, eGFR and Hb measurements were also made at these time points. Statistical analysis was performed using SPSS.

Results: Of the 33 patients (median age 50.32 years), 22 had cirrhosis, with 9 having renal risk factors (diabetes and hypertension). The remainder were neither cirrhotic nor had renal risk factors. Sustained viriologic response (SVR) rates were comparable between the telaprevir and bocepravir groups (75% and 70.5% respectively).

Serum NGAL levels showed no significant differences based on PI, SVR or level of fibrosis. However, in cirrhotics regardless of PI, a statistically significant rise in NGAL levels from TW0 until EOT with subsequent resolution was observed. TW0 NGAL levels >70 ng/ml were associated with >4 g/dl Hb decline at TW12 (PPV 89%) necessitating erythropoetin initiation.

Cystatin C levels were higher at TW0 in cirrhotics vs. non-cirrhotics (933 vs. 791, p = 0.04) but only increased during therapy in cirrhotic patients. Baseline Cystatin C levels (>900 ng/ml) were linked to >20% decline in eGFR by TW12 (PPV 86%). Renin and creatinine levels did not correspond to Cystatin C levels, thus suggesting dehydration was not a contributory factor to these changes.

Conclusions: TW0 Cystatin C levels (>900 ng/ml) can determine which patients will have significant renal dysfuntion during therapy, whilst serum NGAL levels >70 ng/ml identifies those requiring EPO support, regardless of cirrhosis, thus facilitating safer delivery of PI based therapy.

P0854

ESTIMATING THE COST OFFSETS AND IMPACT ON QUALITY-ADJUSTED LIFE EXPECTANCY ASSOCIATED WITH INCREASING SVR IN UK PATIENTS WITH HEPATITIS C-RELATED ADVANCED LIVER DISEASE

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Background and Aims: Most health economic models for hepatitis C consider the value of treating chronically infected patients; however, few have been designed to model treatment in those with advanced liver disease (ALD). Described herein is an adaptation to the MONARCH model to allow modeling of those with ALD with significantly more granularity, including pre- and post-transplant stages. MONARCH ALD was used to estimate the potential benefit of increased efficacy associated with novel direct-acting antivirals (DAAs) over conventional treatment with pegylated interferon-alfa plus ribavirin (PR) when treating patients from the following disease stages: compensated cirrhosis (CC); decompensated cirrhosis (DC); and post-liver transplant fibrosis stage F2 (pLTx-F2).

Methods: A systematic literature review informed the structural framework of the model and key parameters for modeling patients with ALD. Transition rates between health states were obtained from published sources. Costs and mortality estimates were obtained from UK-specific sources. Baseline sustained virologic response (SVR) rates of 30.0%, 14.3% and 45.6% for PR (extracted from published sources) were utilised, with future costs and health benefits discounted at 3.5%. An SVR of 90% was used for comparison to a hypothetical novel DAA. Total complication costs and quality-adjusted life expectancy (QALE) of patients aged 55 with HCV therapy commencing from CC, DC or pLTx-F2 were estimated.

Results: Predicted complication costs and QALEs utilising base case and improved SVR rates are presented in Table 1.

Table 1.

Stage of treatment initiation	Cost of complications (£)			QALE		
	PR	DAA	Cost-offset	PR	DAA	Incremental QALE
CC	32,444	7,801	24,642	8.28	11.23	2.95
DC	104,949	93,230	11,719	7.31	7.96	0.65
pLTx-F2	81,352	47,035	34,317	10.56	12.2	1.63

Conclusions: With the introduction of new therapies with higher rates of SVR, it is likely that significant benefits, in terms of reducing complication costs and increasing QALEs, over conventional therapies will be realised. The greatest cost offset due to the increase in SVR was observed in those that initiated treatment from the pLTx-F2 health state. The greatest improvement in QALE was observed in patients that initiated treatment from the CC health state.

P0855

PHARMACOKINETICS OF ABT-493 AND ABT-530 IS SIMILAR IN HEALTHY CAUCASIAN, CHINESE, AND JAPANESE ADULT SUBJECTS

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Background and Aims: A next generation direct acting antiviral (DAA) combination of ABT-493 (NS3/4A protease inhibitor discovered by AbbVie and Enanta) + ABT-530 (NS5A inhibitor) is being developed for the treatment of chronic hepatitis C infection. This study assessed pharmacokinetics (PK) and safety of multiple oral doses of ABT-493 and ABT-530 administered alone and in combination in healthy Han Chinese, Japanese, and Caucasian subjects.

Methods: This Phase 1, single center, multiple-dose, open-label study consisted of 11 Han Chinese, 12 Japanese, and 12 Caucasian subjects randomized into 2 cohorts with stratification by race (Figure). Intensive PK assessments were performed on Days 1, 7, 8 and 14. PK parameters were estimated by noncompartmental analyses. Safety [adverse events (AEs), clinical labs, vital signs, ECG] was assessed throughout the study.

Results: ABT-493 and ABT-530 exposures (difference ≤32% and ≤58%, respectively) were comparable in Japanese, Chinese and Caucasians when administered alone. When taking DAAs in combination, three ethnic groups also had similar ABT-493 and ABT-530 exposures: the geometric means for ABT-493 C_{max} were 15300, 13900, and 16700 ng/mL, and for AUC were 66000, 49400, and 67500 ng·h/mL, respectively in Caucasian, Chinese, and Japanese; for ABT-530 C_{max} were 289, 288, and 326 ng/mL, and for AUC were 2910, 2570, and 3070 ng·h/mL, respectively in Caucasian, Chinese, and Japanese. Interaction between ABT-493 and ABT-530 were also comparable among ethnicities: on average ABT-493 AUC and C_{max} were increased by 17–61% and 16–30%, respectively, in presence of steady-state ABT-530; ABT-530 AUC and C_{max} were increased to 5.1- to 7.5-fold and 3.1- to 5.0-fold, respectively in presence of steady-state ABT-493. Elimination half-lives of ABT-493 and ABT-530 were similar among Japanese, Chinese, and Caucasian.

Asymptomatic Grade 3 ALT and Grade 2 AST elevation (without concurrent bilirubin elevations) were observed in a Caucasian subject, and 2 additional subjects (Chinese and Caucasian) developed isolated asymptomatic Grade 3 total bilirubin elevations with predominantly indirect fraction. Overall the regimens were well tolerated. There were no serious AEs, premature discontinuations and described laboratory abnormalities normalized/improved following completion of dosing.

	Days 1 to 7	Days 8 to 14
Cohort 1 (N = 18)	ABT-493 700 mg QD	ABT-493 700 mg QD ABT-530 160 mg QD
Cohort 2 (N = 17)	ABT-530 160 mg QD	ABT-493 700 mg QD ABT-530 160 mg QD

Conclusions: Ethnicity does not have much impact on the exposures, safety and tolerability of ABT-493 or ABT-530 alone, or ABT-493 + ABT-530 combination.

P0856

OMBITASVIR/PARITAPREVIR/RITONAVIR AND DASABUVIR WITH RIBAVIRIN (RBV) HAS MILD IMPACT ON HEALTH-RELATED QUALITY OF LIFE (HRQOL) COMPARED WITH PLACEBO DURING 12-WEEK TREATMENT IN TREATMENT-EXPERIENCED ADULTS WITH CHRONIC HEPATITIS C (CHC)

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Background and Aims: Interferon can negatively impact patient HRQoL during CHC treatment. We assessed the HRQoL impact of an interferon-free all-oral CHC therapy – ombitasvir/paritaprevir (identified by AbbVie and Enanta)/ritonavir and dasabuvir with RBV (3D+RBV) – compared with placebo in treatment experienced, non-cirrhotic, Genotype 1 (GT1) adults during 12 weeks of treatment in the Phase 3 trial SAPPHIRE-II.

Methods: Patients were randomized in a 3:1 ratio to 3D+RBV or placebo and treated during a 12-week double-blind period. HRQoL was assessed using the SF-36 v2 Health Survey (SF-36) which was administered at baseline, during treatment, and at end of treatment (EOT) for both treatment groups, and at post-treatment (PT) visits for the 3D+RBV group only. Physical Component Summary (PCS) and Mental Component Summary (MCS) scores were calculated for the SF-36. Summary statistics of change from baseline, including mean and standard deviation (SD), were generated for each visit by treatment group. A repeated measures analysis of covariance (RM-ANCOVA) was carried out for PCS and MCS, respectively, to determine the mean difference in adjusted scores between treatment groups during the 12-week treatment period. The factors in the RM-ANCOVA analysis were treatment group and visit and covariates were baseline PCS score, MCS score, and patient characteristics.

Table: Mean baseline (SD) and change from baseline (SD) HRQoL scores by treatment group

		Baseline	Wk 4	Wk 8	EOT	PT Wk 4	PT Wk 12
PCS	3D+RBV	50.7 (8.46)	−2.2 (6.67)	−3.0 (7.47)	−2.8 (7.74)	0.0 (6.46)	0.8 (6.36)
	Placebo	50.7 (8.03)	−1.2 (4.59)	−0.6 (5.26)	−1.3 (6.27)	n.a.	n.a.
MCS	3D+RBV	50.0 (9.86)	−2.4 (7.91)	−3.9 (9.37)	−3.7 (9.74)	−0.3 (8.41)	0.8 (8.54)
	Placebo	48.6 (11.08)	0.5 (7.55)	−0.5 (7.78)	−0.6 (9.04)	n.a.	n.a.

Results: The analysis included 297 patients on 3D+RBV and 97 on placebo. HRQoL results are summarized in Table. At EOT, greater changes from baseline PCS and MCS scores were observed in the 3D+RBV group (−2.8 in PCS and −3.7 in MCS) compared with the placebo group (−1.3 for PCS and −0.6 for MCS). At PT Wk 12 visit, PCS and MCS scores for 3D+RBV patients were improved over baseline (PCS: +0.8 and MCS: +0.8). Results from RM-ANCOVA demonstrated that the adjusted mean decrements in PCS and MCS scores over the 12 week treatment period in the 3D+RBV group were greater than in the placebo group, and both differences were statistically significant – 1.57 greater decrement in PCS (95% CI, 0.31 to 2.84) and 2.45 greater decrement in MCS (95% CI, 0.83 to 4.07).

Conclusions: During the 12-week treatment period in SAPPHIRE-II, the interferon-free all-oral 3D+RBV regimen had mild impact on patient HRQoL compared with placebo. Post-treatment scores for 3D+RBV showed improvement over baseline.

P0857

98% SVR12 IN KOREAN AND TAIWANESE PATIENTS WITH CHRONIC GENOTYPE 1 HCV INFECTION RECEIVING 12 WEEKS OF LEDIPASVIR/SOFOSBUVIR: RESULTS FROM AN INTERNATIONAL, MULTICENTER PHASE 3 STUDY

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Background and Aims: Approximately half of the patients with chronic hepatitis C virus (HCV) infection in Korea and Taiwan are infected with HCV genotype (GT) 1. The majority of these patients have often failed to respond to prior interferon (IFN)-based therapy. Frequently, patients may be ineligible for, or intolerant of current treatment options. Highly effective, safe and well-tolerated IFN- and ribavirin-free therapies are needed to address the burden of HCV-related liver disease in Korea and Taiwan.

Methods: An open-label, Phase 3 study was conducted to evaluate the efficacy and safety of the ledipasvir (LDV) 90 mg/sofosbuvir (SOF) 400 mg fixed-dose combination (FDC) tablet administered orally, once daily for 12 weeks in Korean and Taiwanese adults with chronic GT1 HCV infection, with and without cirrhosis. There was no upper age limit, we included patients with cirrhosis, no entry restriction applied for neutrophils and the minimum platelet count was 50,000/μL. NS5A and NS5B resistance associated variants (RAVs) were evaluated by deep sequencing. The primary efficacy endpoint was SVR12.

Table 1. SVR12 rates in GT 1-infected patients and difficult-to-treat subgroups

	Korea (N = 93)	Taiwan (N = 85)	Overall (N = 178)
Virologic response (ITT):			
Overall, SVR12, n (%)	92 (99)	83 (98)	175 (98)
On-treatment failure, n (%)	0	0	0
Relapse, n (%)	1 (1)	1 (1)	2 (1)
Withdrew consent, n (%)	0	1 (1)	1 (<1)
SVR12 by subgroup:			
Treatment-experienced (TE), n/N (%)	46/47 (98)	41/43 (95)	87/90 (97)
Cirrhotic, n/N (%)	17/17 (100)	9/9 (100)	26/26 (100)
TE with cirrhosis, n/N (%)	13/13 (100)	4/4 (100)	17/17 (100)

Results: 178 patients were enrolled. The mean (range) age was 54 (20–75) years old, BMI 24 (18–38) kg/m^2, and baseline HCV RNA 6.7 (3.7–7.6) $\log_{10}$ IU/mL. The majority of patients were treatment-experienced (51%), non-cirrhotic (85%), GT1b infected (93%), female (56%), and had IL28B CC genotype (72%). Table 1 shows the overall SVR12 rates. A total of 38 subjects were identified as having baseline NS5A RAVs, of which 37/38 (97%) achieved SVR12. Three (2%) patients failed to achieve SVR12: 2 patients' relapsed and 1 patient withdrew consent. Both relapse patients had detectable NS5A RAVs, but no detectable S282T at the time of relapse. Headache was the only adverse event (AE) reported in ≥10% of patients. Five patients (3%) had treatment-emergent SAEs all unrelated to study drug. Two patients (1%) discontinued therapy due to an AE. No AE leading to

discontinuation occurred in >1 patient. No significant laboratory abnormalities were observed

Conclusions: A single tablet regimen of ledipasvir/sofosbuvir administered once daily for 12 weeks is highly effective and well tolerated in Korean and Taiwanese patients with genotype 1-infection, including those with cirrhosis.

P0858

EARLY HCV RNA DECLINE BY BASELINE CHARACTERISTICS IN HCV INFECTED PATIENTS RECEIVING SOFOSBUVIR-BASED TREATMENT: AN ITALIAN SINGLE CENTER EXPERIENCE

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Background and Aims: In Italy, experience with IFN free regimens is yet mostly related to nominal drug use. Therefore, early virologic response in pts with different genotypes on Sofosbuvir (SOF)-based treatment is available in real life in limited numbers of pts.

Methods: All pts starting SOF-based regimens at our center and expected to have completed at least 4 weeks post treatment at the time of the meeting were evaluated regardless of genotype and treatment regimen. Used regimens included: SOF + PegIFN + ribavirin (RBV), SOF + RBV, SOF + Simeprevir (S), SOF + Daclatasvir (DAC), SOF + ledipasvir (LDV). We assessed HCV RNA at wk 2 and wk 4 by Abbot RealTime (LOQ 12 UI/ml). Liver disease was assessed by both fibroscan and APRI. Univariate and multivariate analysis of baseline characteristics associated with undetectable HCV RNA at week 4 was performed.

Results: Due to the lack of week 2 available HCV RNA results in 6 pts, 89 of 95 starting SOF-based regimens, are analysed here. 54 were NR, 10 PI failure, and the remaining naive. Only 11 patients received PegIFN in combination with SOF + RBV. IFN free regimens were SOF + RBV in 37, SOF + S +/− RBV in 7, SOF + DAC +/− RBV in 37 and SOF + LDV in 7. PI failures received only SOF + DAC. Overall, 67% were male, 85% >45 yr-old, 55% genotype 1, 20% genotype 2, 24% genotype 3 and 1% genotype 4, 80% cirrhotics, 43% with BMI ≥26. Baseline HCV RNA <800,000 IU/ml in 64%, and IL28B CC in 15% were observed. HCV RNA was undetectable or among genotype 2>3>1 both at week 2 ($p = 0.047$) and week 4 ($p = 0.26$). By week 12, all but one, genotype 3 infected, were HCV RNA undetectable (Table). 90% of pts either cirrhotic or not achieved undetectable/or not achieved undetectable/or <LOQ at week 4 ($p = 0.64$). Although not statistically different, week 4 HCV RNA response was lower in previous PI failure than in PegIFN + RBV NR (78% vs 95%, $p = 0.17$)

	HCV RNA undetectable, n/N (%)		
	Week 2	Week 4	Week 12
Genotype 1	19/32 (39%)	29/33 (88%)	33/33 (100%)
Genotype 2	14/15 (93%)	16/16 (100%)	16/16 (100%)
Genotype 3	16/21 (76%)	20/21 (95%)	20/21 (95%)

Conclusions: SOF-based regimens induce wk 4 virologic response regardless of genotype and treatment regimens. The only on treatment failure was related to genotype 3. The vast majority of Genotype 1 PegIFN and RBV NR showed on treatment successful responses after 4 wks of either SOF + PegIFN + RBV or SOF + S.

P0859

GENOTYPE 1 AND 4 CIRRHOTICS: WHAT IS THE SVR RATE IN COMMUNITY CLINICS USING SIMEPREVIR AND SOFOSBUVIR?

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Background and Aims: Combination Simeprevir and Sofosbuvir (SIM/SOF) achieved high SVR rates in Clinical Trials. This combination is currently approved by the FDA and recommended by the AASLD as a treatment option for Genotype 1 infection. The treatment efficacy is not known in patients with cirrhosis in real life experiences. Our aim was to evaluate the safety, tolerability, and efficacy of SIM/SOF in treating chronic Hepatitis C in cirrhotic patients with Genotype 1 and 4 in liver specialized community based clinics. The community clinics were run by nurse practitioners following a standardized protocol.

Methods: 32 patients with liver cirrhosis commenced therapy with Simeprevir 150 mg daily and Sofosbuvir 400 mg daily for 12 weeks. 23 (71.0%) were males with mean age of 61.2±6.4 years. 23 patients (71%) were Peg-IFN treatment experienced. Genotype 1 accounted for 87% of the subjects and 13% had Genotype 4. Baseline platelet levels were $113.4 \pm 46 \times 10^3/\mu L$, albumin 3.7±1.9 g/dL, bilirubin 1.35±1.5 mg/dL and ALT/AST were 82.31±55.1 IU/L and 80.9±70.6 IU/L respectively. 30 patients were Child A; 2 patients were Child B with no clinical signs of liver decompensation. HCV PCR was obtained before, during, and after treatment using commercial assays. Safety data was collected and laboratory tests were performed every 2–4 weeks according to the standard treatment protocol.

Results: 23 (72%) patients achieved HCV below detectable levels by W4. 24/25 (98%) patients completing 12 weeks are negative at end of treatment. SIM/SOF was well tolerated with no signs or laboratory tests indicating liver decompensation. Mean ALT/AST dropped significantly in 68.0% of patients by W4. Platelet counts increased 2%>4% during therapy. SVR12 was achieved in 12/15 (80%) of the patients. 3 patients relapsed within the first 2 weeks after end of therapy.

	Naïve	Treatment experienced
Week 4	n = 5 (71%)	n = 18 (86%)
Week 8	n = 7 (100%)	n = 20 (100%)
Week 12	n = 6 (100%)	n = 18 (95%)
SVR 4	n = 5 (100%)	n = 10 (71%)
SVR 12	n = 4 (80%)	n = 13 (81%)

Conclusions: (1) The combination of SIM/SOF is well tolerated. (2) Not all patients achieve week 4 negativity. (3) Cirrhotic patients who finished 12 weeks of treatment have high SVR12 response rates. (4) All Genotype 4 patients who finished 12 weeks of follow-up achieved viral cure. The remaining patients continue on treatment.

Summary: Though this is a limited study in community clinics, it provides high response rates in a real-life setting of cirrhotic patients using the combination of Simeprevir/Sofosbuvir. Thus far, SIM/SOF has shown to be safe, tolerable, and effective. Data collection continues with more cirrhotic patients being treated with this combination.

P0860

THE IMPACT OF INTERFERON-FREE REGIMENS ON EMPLOYMENT RATE DURING TREATMENT IN PATIENTS WITH CHRONIC HEPATITIS C

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Background and Aims: The current standard-of-care newly approved therapies, Sofosbuvir (Sofo), Daclastavir (Dacla) and Simeprevir (sime) lead to hight rate of sustained response (SVR). However, the impact of these news regimens on unemployment rate is unknown. Purpose: to determine changes in employment status of patients who underwent the new treatments for hepatitis C (HCV) and to identify predictors of employment during treatment.

Methods: 117 patients who had completed HCV therapy (Sofo + ribavirin or sofo + Dacla or sofo+Sime) were consecutively recruited from the referral hospital Beaujon, France between January 2014 and November 2014. A questionnaire was administered prospectively for all patients. Chart review was conducted for labs and biopsy results. A binomial test was conducted to find if antiviral therapy affects on the employment rate. We compared our current truancy rate against the null hypothesis that p = 1. We tested each study charateristic's effect on the employment rate. Our data is categorical. The chi-square test or exact test could be used according the assumption of normality due to the sample size under the null hypothesis that the correlation is 0 to test if each study characteristic has a relationship with the employment rate.
Results: Eighty-nine patients were male (76%), the median age was 53 (range 43–76), 74 (63%) patients worked prior to treatment and 63 (54%) worked during treatment. Five patients (3%) attributed a decrease in work hours to HCV therapy. Six of the seventeen patients who did not return to work with health concerns as the reason. Variables associated with employment during treatment included the following: safety and tolerability during of treatment ($p \leq 0.001$), working conditions ($p = 0.041$), age ($p = 0.001$) and lack of compliance to treatment ($p = 0.08$). Fibrosis stage was not associated with employment ($p = 0.174$). The binomial test is 0.143, indicating that antiviral therapies do not affect employment rates during treatment.
Conclusions: The use of new antiviral therapy seems to be compatible with the maintains employment in a tertiary care referral center. The improved safety and tolerability decreased unemployment rates seen in patients treated regimen based interferon or in general population. This is the first study to our knowledge to focus on the main outcome variable of employment status during HCV treatment. More studies are needed for global assessments of QOL with extrapolation to effects on work productivity.

P0861
EVALUATION OF THE PAN-GENOTYPIC HCV NS3/4A PROTEASE INHIBITOR GS-9857 IN HEALTHY VOLUNTEERS

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Background and Aims: GS-9857 is a pan-genotypic HCV NS3/4A protease inhibitor (PI), in development for the treatment of chronic HCV infection, with potent antiviral activity against HCV genotypes 1–6 and an improved resistance profile compared to previous HCV PIs. In pre-clinical studies, GS-9857 exhibited high metabolic stability, low systemic clearance, and low potential for metabolic drug–drug interactions. This Phase 1 first-in-human study evaluated the safety, tolerability and pharmacokinetics of GS-9857 in healthy volunteers.
Methods: This was a randomized, double-blind, placebo-controlled study with 3 staggered dose-escalation cohorts. Within each cohort, unique healthy subjects were randomized to receive single (SD) and multiple (MD) daily doses (10 days) of GS-9857 (N = 12/cohort) or matching placebo (N = 3/cohort) of 30 mg, 100 mg or 300 mg in the fasted state, with a 6-day washout between SD and MD. Safety was examined throughout the study. SD and MD GS-9857 PK parameters were estimated.
Results: 45 subjects were enrolled and completed the study: 36 subjects received GS-9857 and 9 subjects received placebo. The most frequently reported adverse events (>1 subject) in subjects administered GS-9857 were constipation (n = 2) and diarrhea (n = 2). All treatment emergent AEs were mild in severity. No notable laboratory abnormalities were observed. GS-9857 was absorbed quickly with Cmax occurring 2 to 5 hours post-dose. Across the doses tested, GS-9857 exhibited a median steady-state half-life of 28 to 41 hours. Steady-state GS-9857 exposure was achieved after 7 days of repeated dosing. In general, GS-9857 exposure increased linearly from 30 mg to 300 mg upon SD and MD (Table 1). Accumulation ratios of 2.1 to 4.2 were observed for GS-9857 AUC, Cmax and C24, with slightly greater accumulation (~2-fold) upon multiple dosing of 100 or 300 mg than predicted from single dose data (AUCtau vs. AUCinf).

Table 1. GS-9857 PK parameter summary (N = 12)

PK parameter	30 mg		100 mg		300 mg	
	SD	MD	SD	MD	SD	MD
$AUC_{inf\ or\ tau}$ (h·ng/mL)	56.9 (107)	74.2 (40.7)	195 (92.8)	364 (81.3)	485 (58.1)	1030 (77.3)
C_{max} (ng/mL)	3.7 (56.9)	7.3 (57.6)	12.6 (80.7)	46.0 (126)	34.5 (54.2)	155 (99.6)
$C_{24\ or\ tau}$ (ng/mL)	0.5 (56.5)	1.9 (50.6)	2.0 (123)	5.7 (44.9)	4.4 (67.9)	10.0 (56.2)
$T_{1/2}$ (h), median (Q1, Q3)	–	41.0 (30.1, 46.5)	25.8 (18.4, 39.4)	30.5 (27.3, 32.0)	35.5 (28.8, 46.3)	27.6 (25.1, 34.1)

PK parameters are presented as Mean (%CV) to 3 significant digits as applicable.

Conclusions: GS-9857 was well tolerated as single or multiple daily doses in healthy subjects. The in-vitro antiviral potency, safety and PK profile of GS-9857 supports once-daily dosing for further evaluation of its antiviral activity in HCV-infected subjects.

P0862
FIRST REAL CLINICAL PRACTICE DATA ON SOFOSBUVIR, SIMEPREVIR AND DACLATASVIR WITH HCV-CHRONICALLY INFECTED PATIENTS IN SPAIN: THE HepatiC REGISTRY EXPERIENCE

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Background and Aims: Direct-acting antivirals (DAA) have become elective treatment for HCV chronic infection, especially in patients with advanced liver disease. After phase III trials, sofosbuvir (SOF), simeprevir (SIM) and daclatasvir (DCV) have recently started their use in Spain in different combinations under compassionate regimen. The aim of this study is to analyse the initial experience in Spain on these new treatments in real clinical practice.
Methods: AEEH's HepatiC is a multicentric monitored registry including HCV-infected patients under DAA. Sixty-nine non-liver transplant patients were selected on treatment under compassionate use. The main basal characteristics and safety data regarding SVR at week 4 post-end of treatment (SVR4) were assessed.
Results: Eighty-four percent of the patients had G1 and the others had G3–4. Fibrosis stage was F3/F4 in 96% (F4 88%), and 25% had ascitic decompensation at treatment onset (median MELD 12, 6–21). The most part of the patients (67%) were treatment-experienced, including new DAA (2%). Treatment groups: SOF/PR (n = 4), SOF/R (n = 29), DCV/PR (n = 1), SOF/DCV±R (n = 34), SIM/DCV±R (n = 1). Currently, 20/69 patients have data on SVR4. With complete data pending, global SVR is 80%. SVR4 by group is: SOF/PR, 100% (3/3); SOF/R, 85% (11/13); DCV/PR, 0% (0/1); SOF/DCV±R, 67% (2/3). Patients with ascites at treatment had lower SVR4 (33% *vs* 88%, $p < 0.05$). Albumin levels improved after treatment (3.3 *vs* 3.9 mg/dL, $p = 0.05$), unlike bilirubin (1.75 *vs* 1.15 g/dL, $p = 0.232$) or platelets (69 *vs* 72×10^9/L, $p = 0.264$). Regarding safety, 20% of the patients experienced severe adverse events (SAE) during treatment, mainly liver decompensation (9/14, 64%), transfusion-requiring anemia (4/14, 29%) and grade 3–4 infection (2/14, 14%). Nevertheless, only 9% required discontinuation due to SAE. No association was observer between interferon use and SAE ($p = 0.785$), yet this group is

small (n = 4). Finally, 17% of the patients were liver transplanted on treatment, with available data on SVR4 in 4 of them (SVR4 100%).
Conclusions: With complete data pending, first data in real clinical practice show that new DAA are highly effective even in patients with advanced and decompensated liver disease, improving liver function and being able to prevent post-transplant recurrence. While SAE are still present, DAA discontinuation was necessary in a low proportion of patients. Complete data on SVR12 will be presented, including SOF+DCV and SIM+DCV with and without R, 12 vs 24 weeks.

P0863
"REAL-LIFE"-EXPERIENCE WITH SECOND GENERATION DIRECT ACTING ANTIVIRAL (DAA) TREATMENT IN HCV PATIENTS (N = 149)

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Background and Aims: In 2014, the second generation DAA, Sofosbuvir (SOF), Simeprevir (SMP), and Daclatasvir (DAC) were approved by the authorities. This single center retrospective analysis was conducted to assess the efficacy and safety of 2nd generation DAA treatment in HCV patients.
Methods: 149 consecutive patients infected with HCV were treated with (i) SOF, Ribavirin (RBV) +/− PEG-IFN (n = 55), (ii) SOF, DAC +/− RBV (n = 65), (iii) SOF, SMP +/− RBV (n = 29). 67 patients were treatment naïve, 82 patients had been pretreated either with PEG-IFN/RBV +/− 1st generation protease inhibitors (Telaprevir, Boceprevir), or with SOF/PEG-IFN/RBV (n = 1). Distribution of HCV genotypes (GT): GT 1a: 24%, GT 1b: 42%, GT 2: 3%, GT 3: 22%, GT 4: 6%, GT 6: 1%. Median baseline viral load was 1,335,000 IU/ml HCV RNA. 38% had liver cirrhosis. 10 patients were treated after liver transplantation, 2 of those with a cholestatic recurrence of HCV, one with advanced graft failure. 1 patient was treated post-kidney transplantation.
Results: *Efficacy:* At treatment week (TW) 2, 9% (6/66) were HCV negative, while at TW 4, 69% (59/86) were HCV negative [subgroup analysis TW 4: SOF/RBV +/− PEG-IFN: 80% (32/40) HCV negativity; SOF, DAC +/− RBV: 60% (15/25) HCV negativity; SOF, SMP +/− RBV: 57% (12/21) HCV negativity]. Notably, 49/149 patients already have reached end of treatment, 11 of these patients have reached SVR12.
Safety: 1 patient with relapse after SOF/RBV/PEG-IFN had liver cirrhosis and developed liver failure after relapse, but could be retreated with SOF-DAC, and now is HCV negative again. 1 patient being treated in the course of an advanced graft failure after liver transplantation died due to complications of transplant failure during treatment.
Conclusions: New DAA are highly effective with respect to early viral kinetics. Groups of patients with advanced cirrhosis, or previous treatment failure now can be treated effectively and savely with IFN-free treatment regimens. Most patients will have reached SVR 4, or SVR 12 until March, 2015. These data will be presented at the ILC 2015.

P0864
THE IMPACT OF RIBAVIRIN ON REAL WORLD ADHERENCE AND DISCONTINUATION RATES IN HCV PATIENTS TREATED WITH SOFOSBUVIR + SIMEPREVIR

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Background and Aims: The all-oral combination of sofosbuvir (SOF) and simeprevir (SIM) is used extensively in the United States to treat patients with hepatitis C virus (HCV) infection. A recent analysis of real world data in the US by CVS Health found a four-fold increase in the discontinuation of SOF regimens compared to that in clinical trials. In addition, discontinuation rates varied by type of SOF regimen. Unknown, however, from the CVS analysis is whether the addition of ribavirin (RBV) to SOF+SIM, which raises the pill count of the regimen from 2 pills (2 pills once a day) to 8 pills (2 pills once a day plus 3 pills twice a day), has an impact on adherence and persistence to SOF+SIM. The aim of this study is to examine real-world adherence and discontinuation rates in SOF+SIM patients with and without ribavirin.
Methods: This retrospective study assessed real world adherence (proportion of days covered) and discontinuation (pill gap of >15 days during a 12 week treatment period) for HCV patients in the US initiating SOF-based treatment regimens between December 2013 and March 2014. Analyses were conducted using MarketScan US commercial pharmacy claims and enrollment data for December 2013 through June 2014. Two SOF-based treatment regimens were analyzed: SOF+SIM and SOF+SIM+RBV. Index date was first fill date for SOF. Inclusion criteria included continuous enrollment from the month of index date through the following three months. An exclusion criterion was an 84 day supply of SOF on initial fill. Statistical analyses were conducted using population proportion z-tests. Generalized linear modeling was used to adjust adherence by age, gender and copay (includes copays for SOF, SIM and RBV).
Results: Patient sample size was 286 for SOF+SIM and 65 for SOF+SIM+RBV. The mean age and percent male were not statistically different between cohorts – 56.6 years and 65.0% male for SOF+SIM and 56.7 years and 72.3% male for SOF+SIM+RBV. Differences in unadjusted adherence and discontinuation rates between SOF+SIM (90.6%, 19.2%) and SOF+SIM+RBV (88.2%, 21.5%) were not statistically significant ($p > 0.05$). After adjusting for age, gender and copay, adjusted adherence was 90.2% for SOF+SIM and 87.7% for SOF+SIM+RBV ($p = 0.2915$).
Conclusions: These findings indicate that, in spite of the greater number of pills and more frequent dosing, adding ribavirin to an all-oral regimen of SOF+SIM does not appear to impact HCV medication adherence or persistence in hepatitis C patients.

P0865
THE USE OF SOFOSBUVIR BASED REGIMENS AMONGST TREATMENT NAIVE AND EXPERIENCED PATIENTS WITH ADVANCED FIBROSIS/CIRRHOSIS: REAL WORLD RESULTS FROM A DIFFICULT TO TREAT COHORT

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Background and Aims: Phase 3 trials of Sofosbuvir (Sof) have included patients with cirrhosis at a rate of 17–23%, however, trial inclusion/exclusion criteria may make outcomes less reproducible in routine clinical care. Data is lacking for GT1 treatment experienced (TE) patients, and data for treating GT3 patients with pegylated interferon (IFN) based regimens limited. We sought to establish the tolerability and effectiveness of Sof based regimens to treat naive and TE patients with advanced fibrosis/cirrhosis in routine practice.
Methods: We analysed the Scottish HCV Database to identify patients with a liver stiffness ≥9.5 kPa starting a Sof based regimen [with Ribavirin (RBV) +/− IFN] in Glasgow treatment centres. Data were obtained on demographics, liver stiffness, cirrhosis status, treatment status, baseline laboratory measurements, Child's score, presence of varices, and history of drug and alcohol abuse. Data were compared with phase 3 trial inclusion/exclusion criteria.

Baseline viral load, week 4 PCR, EOTR and SVR12 were recorded. Discontinuation rates and reasons were recorded.

Results: 122 patients, were treated with either Sof/IFN/RBV (n = 107) or Sof/RBV (n = 15). Baseline characteristics are summarised per treatment regimen in Table 1. 97 (79.5%) patients were cirrhotic, with a median liver stiffness of 20.8 (IQR 14.8–35.5). The majority were GT3 (69, 56.5%) or GT1 (45, 36.8%). Around half (47.6%) were TE, including 9 Protease Inhibitor failures. 65 (53.2%) patients would have failed at least one trial exclusion criterion [37 (30.3%) of the cohort due to baseline labs, 30 (24.6%) recent drug/alcohol misuse, 1 (0.8%) schizophrenia].

Table 1.

Patient characteristics	Sof/IFN/Rib (n = 107)	Sof/Rib (n = 15)
Mean age (range)	48 (23–76)	50 (39–69)
Male (%)	83 (77.5)	8 (53.3)
Previous IVDU (%)		
Never	43 (40.1)	5 (33)
>12 months	51 (47.6)	9 (62)
Within last 12 months	10 (9.3)	1 (5)
Unknown	3 (2.8)	–
Former alcohol excess (%)	42 (38.9)	8 (53.3)
In 12 months prior to Tx	18 (16.8)	1 (5)
Treatment experienced (%)	50 (46.7)	10 (66.6)
Median viral load (IQR)	5.69 (5.22–6.31)	5.58 (4.64–6.37)
Genotype (%)		
1	43 (40.1)	2 (13.3)
2	1 (0.9)	3 (20)
3	59 (55.1)	10 (66.6)
4	4 (3.7)	–
Cirrhosis (%)	83 (77.5)	14 (93.3)
Child A/B/C	76 (91.5) / 7 (8.4) / –	7 (50) / 7 (50) / –
Platelets <90	22 (26.5)	3 (21.4)
Platelets <50	–	3 (21.4)
Varices	16 (19.2)	6 (42.9)
Median LSM (IQR)	20.9 (14.3–34.3)	30.4 (25.1–37.5)

To date 1 patient has been lost to follow up and one stopped due to non compliance. 1 patient died suddenly at home (post mortem pending, not clearly related to treatment). No other patient discontinued due to adverse events.

106 patients had week 4 results, of whom 30 (28.3%) had undetectable RNA (RealTime HCV, Abbott Laboratories). 45 (42%) were detectable below the lower limit of quantification (LLOQ) of 12 IU/ml, and 31 (29.%) had quantifiable RNA (median 24 IU/ml, IQR 15–58). 34/35 (97.1%) patients were PCR negative at the end of treatment, with one positive.

Conclusions: Patients with advanced fibrosis/cirrhosis and unfavourable baseline characteristics can be treated with Sofosbuvir based regimens. High numbers of such patients had detectable HCV RNA at week 4, however, this does not appear to impact on end of treatment response. SVR12 data will be presented.

P0866

COMPARATIVE EFFICACY AND TOLERABILITY OF DACLATASVIR + SOFOSBUVIR VERSUS SOFOSBUVIR + RIBAVIRIN FOR HEPATITIS C GENOTYPE 3: A MATCHING-ADJUSTED INDIRECT COMPARISON

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Background and Aims: To compare the efficacy and tolerability of 12 weeks of daclatasvir and sofosbuvir (DCV+SOF) versus 24 weeks of SOF and ribavirin (R) in both treatment-naïve and treatment-experienced patients infected with chronic hepatitis C (HCV) genotype 3.

Methods: A systematic literature review was conducted to identify Phase 3 trials of SOF+R in genotype 3 patients with HCV. One trial with the approved regimen of SOF+R (VALENCE) was identified for the comparison with the DCV+SOF trial (ALLY-3). Individual patient data from ALLY-3 were available (n = 144); published summary data were extracted from the 24-week SOF+R-treated arm from VALENCE (n = 250). To adjust for cross-trial differences, ALLY-3 patients were weighted to match all available summary baseline characteristics reported in both ALLY-3 and VALENCE; specifically, age, BMI, gender, race, ethnicity, HCV RNA level, alanine aminotransferase level, IL28B genotype, cirrhosis status, prior treatment status, and response type to prior treatment were included in the analysis. Sustained virologic response (SVR) at week 12 post-treatment (overall and stratified by prior treatment experience), discontinuation due to adverse events (AEs), and rates of specific AEs (with an incidence of >10%) were compared between the treatments.

Results: After adjustment for baseline characteristics, SVR12 was comparable in the overall, treatment-naïve, and treatment-experienced populations in ALLY-3 and VALENCE (Table 1). In the overall population, the rate of discontinuation due to AEs was similar in patients treated with DCV+SOF and patients treated with SOF+R. Rates of asthenia, cough, dry skin, dyspnea, irritability, nasopharyngitis, and pruritus were significantly lower in patients treated with DCV+SOF compared to patients treated with SOF+R. Rates of all other assessed AEs were similar between the treatment regimens.

Table 1. Comparison of SVR12 rates in overall, treatment-naïve, and treatment-experienced patients

Group	SVR 12 (%)		
	DCV + SOF		SOF + R
	Pre-adjustment	Post-adjustment	
Overall	89.6	88.8	85.2
Treatment-naïve patients	90.1	96.4	94.3
Treatment-experienced patients	88.4	83.2	78.6

Conclusions: After adjustment for cross-trial differences in baseline characteristics, 12 weeks of DCV+SOF was associated with similar rates of SVR12 in the overall, treatment-naïve, and treatment-experienced populations, and with similar or lower rates of discontinuation and AEs in the overall population, compared with 24 weeks of SOF+R in HCV genotype 3 patients.

P0867

EFFICACY EVALUATION OF 24 WEEK SOF + RBV IN A HETEROGENEOUS, REAL-WORLD POPULATION OF GENOTYPE 3 HCV PATIENTS; DATA FROM THE TRIO NETWORK

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Background and Aims: HCV genotype 3 has proven difficult to treat in clinical trials and understanding the therapies used in a heterogeneous real-world population and the outcomes of these treatments are important for patients and physicians. Trio Health is a disease management company that works in partnership with academic medical centres, community physicians and specialty pharmacies in the US to optimize care for Hepatitis C. Data obtained through the Trio Health program were used to evaluate 24 week sofosbuvir plus ribavirin (SOF + RBV) for HCV genotype 3 patients.

Methods: Data were collected from Rx records through the Trio Platform in partnership with specialty pharmacies. Analyses were limited to 96 patients who initiated a 24 week course of SOF + RBV between December 2013 and March 2014. These 96 patients were

treated in 35 practices, 17 of which were community based and 18 that were academic.
Results: Mean age was 54 with 11% of patients 65 years or older, 56% male and mean BMI 30.0. Of the 96 patients, 7% were Asian, 3% black, 7% Hispanic and 68% white. HIV co-infection was present in 7% of the population. Mean lab measures at baseline were AST 89 IU/l, ALT 97 IU/l, Hb 14.2 mg/dl, and platelets 158K/ul (range 38K–429K). Baseline Viral load >6MM IU/ml was seen in 15% of patients and ≤800K IU/ml was present in 41% of patients. 39% of patients were previously treated, 41% of whom were null responders. Cirrhosis was present in 30% of patients. Treatment evaluation indicates an overall discontinuation rate of 9%.
Conclusions: An examination of 24 week SOF + RBV in a heterogeneous population with genotype 3 HCV is underway; SVR results will be available at the meeting.

P0868
MODELING COST-EFFECTIVENESS DURING ANTIVIRAL THERAPY FOR CHRONIC HEPATITIS C IN REAL-LIFE PRACTICE BY GENETIC TESTS AND VIRAL KINETIC PROFILE

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Background and Aims: The association between host (IL28B/ITPA SNPs) and viral (genotype and kinetic) are determining factors for sustained virologic response (SVR). We propose a novel model to identify subjects with favorable profile of response and drug-tolerance to tailor therapy strategies, especially with DAAs, based on SVR-probabilitity (pSVR) at week 4 of therapy (4WT) (lead-in phase).
Methods: Prospective-observational study on 1194 cases (773M/421F;aged 46±10 yrs) with chronic hepatitis C, that underwent to determination of: (a) multiplex PCR for detecting IL28B and ITPA SNPs (GeneQuality IL28B-ITPA, AB Analitica) (b) quantitative HCV-RNA test (VL), (c) calculation of per-SVR costs (pharmacoeconomics model).
Results: The HCV1–4 (688 cases) and HCV2–3 (506 cases) IL28B-ITPA SNPs distribution were: rs12979860-CC (29%) and TT (16%); rs8099917-TT (50%) and GG (7%); rs7270101-AA (78%) and CC (2%); rs1127354-CC (87%) and AA (2%). By multivariate analysis, the: VL reduction (>3LogUI/mL) at 4WT, IL28B wild-type, genotype, disease stage, therapy duration/intensity and age were predictive of SVR, while the ITPA variant, was strongly associated with a lower Hb-reduction (<30 mg/dL) at 4WT. Different pSVR groups allowed to determine cost-effectiveness of therapy strategies (table 1) with 86% specificity and 78% sensitivity.

Model at 4WT pSVR (%)	Treated cases (%)	Strategy	SVR cases (%)	Cost (€)/SVR
>60	654 (55)	Continue Peg-IFN + RBV	551 (84)	6,289
40–60	184 (15)	Add BOC, TEL or SOF	140 (76)	31,540
20–40	150 (13)	Add BOC, TEL or SOF	70 (47)	51,400
0–20	206 (17)	Stopping rule	12 (6)	49,878

Conclusions: Our pharmacoeconomic model allows the choice of the best cost-effectiveness strategy to: apply a stopping-rule, add DAAs, or continue Peg-IFN+RBV in cases with low, moderate or high pSVR, avoiding futile therapy and optimizing costs.

P0869
EARLY VERSUS DELAYED USE OF SOFOSBUVIR PLUS PEGINTERFERON/RIBAVIRIN THERAPY IN FIBROSIS PATIENTS WITH HEPATITIS C VIRUS: A COST-EFFECTIVENESS ANALYSIS

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Background and Aims: The administration of treatment in patients with chronic hepatitis C virus (HCV) in different disease stages is associated with a variation in the therapy's effectiveness. The aim of the analysis was assessed the cost-effectiveness of sofosbuvir combined with peginterferon alfa-2a and ribavirin (SOF/PEG-IFN/RBV) at early versus delayed fibrosis disease stage, in previously untreated patients with HCV genotype 1, 52 years aged, from the Spanish National Health System perspective.
Methods: A Markov model with ten health states was developed to compare lifetime cost and outcomes (life-years gained-LYG and quality-adjusted life-years-QALY) of two treatment strategies: early SOF/PEG-IFN/RBV at mild-moderate fibrosis (F2–F3) or delayed treatment at compensated cirrhosis (F4). The efficacy was measured as sustained virology response (SVR) at 12-weeks after the end of therapy (NEUTRINO study). Annual transition probabilities, disease management costs and utilities were obtained from literature. Only direct cost (pharmaceutical and disease cost by health state) were considered. Drug cost for the SOF/PEG-IFN/RBV 12-weeks regimen was calculated with the publicly available local ex-factory prices with applicable mandatory discounts for marketed drugs. Costs were expressed in Euro (€) 2014. A 3% annual discount rate was applied to cost and outcomes. Probabilistic sensitivity analysis (PSA) was performed.
Results: Early SOF/PEG-IFN/RBV therapy at F2–F3 was more effective (19.12 LYG and 14.14 QALY) than delayed administration at F4 (16.36 LYG and 9.27 QALY). In a 1,000 patient cohort, SOF/PEG-IFN/RBV at F2–F3 avoided 66 cases of decompensated cirrhosis, 60 hepatocellular carcinoma and 4 liver-transplantation compared to delayed therapy in F4 patients. Total cost of early therapy at F2–F3 (€56,012.51) was lower than the cost of delayed treatment in F4 patients (€61,767.92). Early versus delayed therapy was a dominant strategy (more effective and less costly). In PSA, early use of SOF/PEG-IFN/RBV remained dominant in 100% of simulations.
Conclusions: Compared to delayed administration of therapy at F4, initiating SOF/PEG-IFN/RBV treatment at early disease stages (F2–F3) reduced the incidence of new cases of liver-disease complications and it was associated to cost savings for the Spanish National Health System in previously untreated genotype 1 HCV-infected patients.

P0870
ESTIMATING THE COST-EFFECTIVENESS OF 12 WEEKS OF TREATMENT WITH DACLATASVIR + SOFOSBUVIR IN PATIENTS CHRONICALLY INFECTED WITH HCV GENOTYPE 3

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Background and Aims: Chronic infection with hepatitis C virus (HCV) genotype 3 is associated with increased rates of disease progression and is considered to be difficult to treat. Further, historically, patients that are ineligible for or intolerant to interferon had no available treatment option. The objective of this investigation was to compare the cost-effectiveness of two all-oral, interferon-free treatment regimens that have recently been licensed

for the treatment of patients with HCV genotype 3: daclatasvir plus sofosbuvir (DCV+SOF) and sofosbuvir plus ribavirin (SOF+RBV). A comparison to no treatment was also made for those that are ineligible for or intolerant to interferon.

Methods: A published Markov model was used to estimate the cost-effectiveness of interferon-free HCV genotype 3 treatment regimens over a lifetime horizon. It was assumed patients were evenly distributed across fibrosis stages F0–F4 upon treatment initiation. Patients progress from chronic infection through disease stages using published transition rates. Disease state costs were obtained from 2013 UK data and discounting was set to 3.5%. The weekly costs of DCV, SOF and RBV were £2,038.13, £2,915.24 and £66.95, respectively. Clinical inputs were obtained from a matching-adjusted indirect comparison (MAIC) of the ALLY-3 and VALENCE studies for DCV+SOF and SOF+RBV, which adjusts for baseline differences between trials.

Results: Predicted incremental costs, quality-adjusted life years (QALYs) and cost-effectiveness results are presented in Table 1.

Conclusions: Historically, HCV genotype 3 patients that were unable to receive interferon had no available treatment option. Based upon a willingness-to-pay threshold of £20,000/QALY, 12 weeks of DCV+SOF appears a cost-effective treatment option for patients chronically infected with HCV genotype 3 in all scenarios. When compared to 24 weeks of SOF+RBV, the DCV+SOF regimen results in improved quality of life and reduced total costs.

Pop.	Regimen	Incremental		ICER (£) versus DCV+SOF
		Cost (£)	QALY	
N	DCV+SOF		–	
	SOF+RBV	−12,963	0.13	Dominant [a]
E	DCV+SOF		–	
	SOF+RBV	−13,761	0.24	Dominant [a]
I	DCV+SOF		–	
	SOF+RBV	−13,442	0.2	Dominant [a]
	No treatment	31,815	4.12	7,728

E, Experienced; I, Interferon-ineligible/intolerant; N, Naïve; Pop., Treatment population.

[a] DCV+SOF is associated with improved quality of life and reduced total costs.

P0871

REAL LIFE EXPERIENCE WITH INTERFERON/RIBAVIRIN-FREE ANTIVIRAL TREATMENT IN RENAL TRANSPLANT RECIPIENTS AND ENDSTAGE RENAL DISEASE-PATIENTS ON DIALYSIS INFECTED WITH HEPATITIS C VIRUS

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Background and Aims: Since introduction of all-oral interferon-free treatment-regimens for hepatitis C virus infection (HCV), former “difficult to treat” patient-groups, like end-stage renal disease (ESRD)-patients or patients after renal transplant (NTx) are focused on recently, as treatment of those represented an area of unmet therapeutically need. As real-life data of sofosbuvir-based all-oral treatment-regimens is scarce, we investigated on-treatment viral response (otVR) and clinical profiles in ESRD- and NTx-patients.

Patients and Methods: Fifteen patients with chronic HCV-infection (2 GT1; 2 GT1a, 7 GT1b; 1 GT3a; 1 GT4; 1 GT4a/c/d; 1 GT1b/3a; Cirrhosis: 8/53%) after NTx (11/73%) or on dialysis (5/33%) due to ESRD are being treated with sofosbuvir (SOF)/daclatasvir (DCV; N = 9), SOF/simeprevir (SIM; N = 5) or DCV/SIM (N = 1); 24 h-dosage: N = 14/93%, 48 h-dosage: N = 1/7% patient(s).

Viral-load was measured by Abbott RealTime HCV-quantitative assay (lower limit of quantification [LLOQ]: 12 IU/ml); treatment-response (defined as: TND [target not detected) evaluated at weeks: 4/8/12(EoT)/16/24(EoT)/SVR4/SVR12. Continuous variables were reported as mean±SD or median (interquartile range).

Results: 15 patients (male: 11; age: 52.4±13.4 [31–71] years mean±SD [range]) were evaluated. On-treatment response was: week 4 – TND: 4/36.4%, LLOQ: 5/45.5%; week 12 – TND: 5/100%; EoT – TND: 5/100%. SVR 12: 1. Treatment-duration was reduced in 1 patient to 12 weeks due to worsening of renal function (creatinine: BL: 3.01/WE12: 4.16 mg/dl; otVR: WE4: LLOQ; EoT: TND). One SOF/SIM-patient developed pancytopenia at week 7; as a result treatment-regimen had to be re-evaluated and switched from 24 h- to 48 h-dosage with consecutive increase in HCV RNA (week 4: LLOQ – week 8: 200 IU/ml). No patient required dose-adjustment of immuno-suppressive treatment or discontinuation due to further (severe) adverse events (clinical or laboratory) as far. Final SVR12-results, safety-data as well as subgroup-analyses will be presented.

Conclusions: On-treatment virological response rates with interferon free therapy-regimens in ESRD- and renal transplant-patients are promising and resulted in rapid viral on-treatment suppression.

Overall SOF-based treatment-regimens were safe in patients with ESRD or after NTx; no interactions with immuno-suppressive regimens were observed.

P0872

EARLY VIRAL KINETICS DO NOT DIFFER IN PATIENTS WITH VARYING DEGREES OF FIBROSIS AND CIRRHOSIS IN THE SOLAR 1 TRIAL

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Background and Aims: Ledipasvir/sofosbuvir (LDV/SOF) + RBV for 12 or 24 weeks led to high SVR rates in patients with genotype 1 or 4 HCV and advanced liver disease or those who were post liver transplantation. We analyzed the viral kinetics in decompensated and post-transplant patients to determine whether on-treatment response varied by patient population.

Methods: Six groups of patients with HCV genotypes 1 or 4 and decompensated liver disease or who were post liver transplantation were randomized to receive 12 or 24 weeks of LDV/SOF+RBV treatment: patients without transplant and either (1) Child–Pugh–Turcotte (CPT) B cirrhosis, or (2) CPT C cirrhosis; or patients who have undergone transplantation and who were either (3) without cirrhosis (F0 to F3), (4) CPT A cirrhosis, (5) CPT B cirrhosis, or (6) CPT C cirrhosis. HCV RNA was measured using the Roche COBAS® Ampliprep®/Cobas TaqMan HCV Test, Version 2.0 (CAP/CTM HCV v2.0) with a lower limit of quantification (LLOQ) <15 IU/mL detected or HCV RNA not detected.

Table (abstract P0872).

	Advanced liver disease pre-transplantation (N = 108)		Post-transplantation (N = 223)			
	CPT B (N = 59)	CPT C (N = 49)	F0–F3 (N = 111)	CPT A (N = 51)	CPT B (N = 52)	CPT C (N = 9)
Overall SVR12, n/N (%)	50/57 (88)	37/42 (88)	108/111 (97)	49/51 (96)	37/44 (84)	5/8 (63)
Week 2 <LLOQ, n/N (%)	23/59 (39)	19/49 (39)	50/110 (45)	16/51 (31)	13/51 (25)	3/9 (33)
Week 2 TND, n/N (%)	7/59 (12)	5/49 (10)	28/110 (25)	6/51 (12)	6/51 (12)	0/9 (0)
Week 4 <LLOQ, n/N (%)	49/59 (83)	45/49 (92)	98/110 (89)	43/51 (84)	41/51 (80)	9/9 (100)
Week 4 TND, n/N (%)	25/59 (42)	28/49 (57)	69/110 (63)	24/51 (47)	24/51 (47)	5/9 (56)
Week 6 <LLOQ, n/N (%)	59/59 (100)	47/48 (98)	108/110 (98)	50/50 (100)	51/51 (100)	9/9 (100)
Week 6 TND, n/N (%)	51/59 (86)	42/48 (88)	97/110 (88)	44/50 (88)	45/51 (88)	8/9 (89)

Results: The percent of patients who were LLOQ by Weeks 2, 4 and 6 is shown in the table.
Conclusions: 12 or 24 weeks of treatment with LDV/SOF+RBV was highly efficacious in patients with advanced liver disease or who were post transplantation. The majority of subjects achieved HCV RNA target not detected (TND) at Week 4. Early on-treatment viral kinetics were similar among patient groups: advanced liver disease CPT class B or C, and in patients post transplantation with varying degrees of fibrosis and cirrhosis. An analysis of the relationship between viral decline at on-treatment weeks 2, 4, and 6, presence of NS5A RAVs, and relapse will be presented.

P0873
OMBITASVIR/PARITAPREVIR/RITONAVIR AND DASABUVIR WITH RIBAVIRIN (RBV) HAS MINIMAL IMPACT ON HEALTH-RELATED QUALITY OF LIFE (HRQOL) COMPARED WITH PLACEBO DURING 12-WEEK TREATMENT IN TREATMENT-NAÏVE ADULTS WITH CHRONIC HEPATITIS C (CHC)

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Background and Aims: Interferon can negatively impact patient HRQoL during CHC treatment. We assessed the HRQoL impact of an interferon-free all-oral CHC therapy – ombitasvir/paritaprevir (identified by AbbVie and Enanta)/ritonavir and dasabuvir with RBV (3D+RBV) – compared with placebo in treatment naïve, non-cirrhotic, Genotype 1 (GT1) adults during 12 weeks of treatment in the Phase 3 trial SAPPHIRE-I.
Methods: Patients were randomized in a 3:1 ratio to 3D+RBV or placebo and treated during a 12-week double-blind period. HRQoL was assessed using the SF-36 v2 Health Survey (SF-36) which was administered at baseline, during treatment, and at end of treatment (EOT) for both treatment groups, and at post-treatment (PT) visits for the 3D+RBV group only. Physical Component Summary (PCS) and Mental Component Summary (MCS) scores were calculated for the SF-36. Summary statistics of change from baseline, including mean and standard deviation (SD), were generated for each visit by treatment group. A repeated measures analysis of covariance (RM-ANCOVA) was carried out for PCS and MCS, respectively, to determine the mean difference in adjusted scores between treatment groups during the 12-week treatment period. The factors in the RM-ANCOVA analysis were treatment group and visit and covariates were baseline PCS score, MCS score, and patient characteristics.
Results: The analysis included 473 patients on 3D+RBV and 158 on placebo. HRQoL results are summarized in Table. At EOT, greater changes from baseline PCS and MCS scores were observed in the 3D+RBV group (−1.3 in PCS and −3.7 in MCS) compared with the placebo group (+0.7 for PCS and −2.0 for MCS). At both PT visits, PCS and MCS scores for 3D+RBV patients were improved over baseline (PCS: +0.5 at week 4 and +1.4 at week 12; MCS: +0.3 at week 4 and +2.0 at week 12). Results from RM-ANCOVA demonstrated that the adjusted mean decrements in PCS and MCS scores over the 12 week treatment period in the 3D+RBV group were greater than in the placebo group, but neither difference was statistically significant – 0.98 greater decrement in PCS (95% CI, −0.11 to 2.07) and 1.39 greater decrement in MCS (95% CI, −0.00 to 2.77).
Conclusions: During the 12-week treatment period in SAPPHIRE-I, the interferon-free all-oral 3D+RBV regimen had minimal impact on patient HRQoL compared with placebo. Post-treatment scores for 3D+RBV showed improvement over baseline.

P0874
OUTCOMES OF 12-WEEK THERAPY WITH SIMEPREVIR + SOFOSBUVIR +/− RIBAVIRIN (SMV+SOF±RBV): A META-ANALYSIS OF 7 STUDIES AND 715 HCV GENOTYPE 1 (HCV-1) PATIENTS

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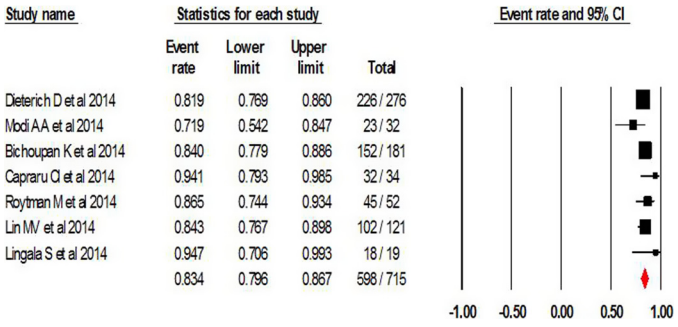
Background and Aims: SVR12 with 12-week SMV+SOF±RBV therapy was shown to be high for HCV GT1 patients in COSMOS study, but its generalizability is limited due to small sample size in a highly controlled clinical trial. Less is known about the effectiveness of the regimen in a real-life setting. Our goal was to perform a meta-analysis of real-world data evaluating the effectiveness of SMV+SOF±RBV.
Methods: A comprehensive literature search for 'simeprevir' in MEDLINE and major liver meetings in 2014 (AASLD, APASL, DDW, EASL, and World Transplant Congress) was performed and data was extracted in November 2014 by two authors using a case report form (BY and NN) with discrepancies resolved by consensus with a third author (MN). We included studies with SVR12 data that had ≥5 HCV GT1 patients treated with SMV+SOF±RBV for 12 weeks. Those who had prior liver transplantation or were co-infected with HIV or HBV were excluded. Primary endpoint was pooled effect sizes for SVR12 with random effects modeling. To evaluate study heterogeneity, Cochrane Q-test (p-value <0.10) with I^2 statistic (>50%) was determined.
Results: Seven studies with a total of 715 HCV GT1 patients were included. All were non-randomized cohorts. The majority of patients were male (range 57–81%). Many had advanced fibrosis (range 45–64%). Overall rate of SVR4 was 91.6% (CI: 87.5–94.5%) and SVR12 was 83.4% (CI: 79.6–86.7%). Three studies had SVR12 data sub-grouped by severity of fibrosis (n = 296, 147 with mild fibrosis and 149 with advanced fibrosis). Definition of advanced fibrosis was variable and included METAVIR F3–F4 or cirrhotic patients. SVR12 rate was 89.7% (CI 79.0–95.3%) in patients with mild fibrosis and 76.2% (CI 68.6–82.4%) in advanced fibrosis. By direct comparison, a trend was found favoring SVR12 for patients with mild fibrosis over advanced fibrosis (OR 2.66, CI 0.816–8.650; *p*-value = 0.105). Data was insufficient to allow evaluation of adverse events or subanalysis for outcomes of SMV+SOF with and without RBV or treatment naïve and experienced patients.

Table (abstract P0874).

Author, year	Setting	Collaboration	Intention-to-treat analysis	Patients with SVR12 data	Age (years)	Male, N (%)	Advanced fibrosis, N (%)
Dietrich D et al., 2014	Mixed	Multicenter	Yes	276	Mean 59	181 (57)	145 (45)
Modi AA et al., 2014	Community	Multicenter	Yes	32	Median 59	34 (76)	–
Bichoupan K et al., 2014	University	Single center	No	181	Median 62	-	96 (53)
Capraru CI et al., 2014	University	Single center	No	34	Mean 57.7	31 (66)	–
Roytman M et al., 2014	University	Single center	No	52	Mean 61.5	63 (64)	63 (64)
Lin MV et al., 2014	University	Multicenter	No	121	Mean 57.7	103 (70)	84 (57)
Lingala S et al., 2014	University	Single center	No	19	Mean 59	16 (64)	–



Conclusions: Pooled rate of SVR12 was approximately 83% in SIM+SOF±RBV for 12 weeks based on cohort studies from real-world settings which include a large proportion of patients with advanced fibrosis and/or prior treatment failure and slightly lower than reported rated from clinical trial, suggesting the importance of examining treatment effectiveness in diverse real-world patients in addition to treatment efficacy seen in highly selected clinical trial patients.

P0875

PREDICTORS OF SUSTAINED VIRAL RESPONSE TO 4–6 WEEK DURATION THERAPY WITH LEDIPASVIR + SOFOSBUVIR + GS-9451 +/− GS-9669 IN EARLY AND ADVANCED FIBROSIS (NIH/UMD SYNERGY TRIAL)

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Background and Aims: Directly-acting antivirals (DAA) have simplified the treatment of hepatitis C into a highly efficacious regimen delivered in as little as 6 weeks. However, limited data exists to define minimum duration of therapy or applicability in advanced liver disease. The aim of the current study is to further eluciate treatment length and predictive factors of response in two cohorts: LDV/SOF + GS-9451 +/− GS-9669 for four weeks in treatment naïve, early fibrosis patients; and LDV/SOF + GS-9451 for six weeks in advanced fibrosis patients, both treatment naïve and experienced.
Methods: In this single-center, open label, phase 2a trial, 100 HCV monoinfected participants were sequentially enrolled into three treatment arms. Treatment naive patients with F0–F2 fibrosis received LDV/SOF + GS-9451 (n = 25) or LDV/SOF + GS-9451 + GS-9669 (n = 25) for 4 weeks. Patients with F3–F4 fibrosis received LDV/SOF + GS-9451 for 6 weeks (n = 25 treatment naïve, n = 25 IFN-treatment experienced). HCV RNA was measured using Roche COBAS Taqman v2.0 assay with a LLOQ of 25 IU/ml. Primary endpoint was sustained virologic response defined as HCV RNA BLLOQ 12 weeks post-treatment (SVR12). Pretreatment serum samples were analyzed for resistance-associated variants (RAV) in the early stage group.
Results: In the early fibrosis cohort, 9/25 (36%) patients receiving LDV/SOF + GS-9451 achieved SVR12, and 5/25 (20%) receiving LDV/SOF + GS-9451 + GS-9669 achieved SVR12. One patient in each group was lost to follow up. Male gender ($p = 0.03$) and baseline HCV VL ($p < 0.0001$) were risk factors for viral relapse in univariate analysis. No patient with a pre-treatment HCV viral mutant conferring >5 fold resistance achieved SVR. In the advanced fibrosis cohort, 22/22 (100%) achieved EOT viral suppression. 6/11 (54.5%) treatment naïve and 5/9 (55.6%) treatment experienced patients achieved SVR12. Data collection and sequencing in this cohort is ongoing, and complete data will be presented.
Conclusions: In this small cohort study, DAA-based treatment for four and six weeks in early and advanced fibrosis, respectively, was effective in select patients. Female patients and those with a low baseline HCV VL were more likely to achieve SVR12. Short treatment durations were not effective in early fibrosis patients with baseline high-level resistance mutants. Larger studies are required to further characterize the biologic correlates in this unique group of responders to short duration therapy, with critical implications on both an individual and public health level.

P0876

SAFETY AND EFFICACY OF SIMEPREVIR/SOFOSBUVIR IN HEPATITIS C (HCV) INFECTED PATIENTS WITH VARYING SEVERITY OF CIRRHOSIS: COMPARISON TO MATCHED UNTREATED AND PROTEASE INHIBITOR (PI)-TRIPLE THERAPY TREATED CONTROLS

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Background and Aims: All-oral therapy offers safety advantages for patients with decompensated cirrhosis. The risks/benefits of simeprevir (SIM) plus sofosbuvir (SOF) in patients with Child–Pugh (CP) B/C cirrhosis are unknown. We aim to assess safety and sustained virologic responses (SVR) of SIM+SOF±ribavirin (RBV) in CP-B/C vs CP-A cirrhosis and to compare results to matched untreated and PI (telaprevir or boceprevir)-triple therapy treated controls.
Methods: Multicenter cohort of adults with HCV genotype 1 cirrhosis treated with SIM+SOF±RBV who have completed ≥4 weeks of therapy (n = 160). Safety and SVR12 outcomes by CP-B/C vs CP-A were examined. Up to 3 untreated and PI-triple therapy

treated controls matched on site, age±5 yrs, CP class (A vs B/C) and model for end-stage liver disease score (MELD) ±2 were randomly selected.

Results: SIM+SOF±RBV (35%/65%) was used for goal duration of 12 weeks; 36% had CP-B/C and 64% CP-A cirrhosis. Cohort median age was 62 yrs (IQR 58–65), with 39% female, 11% Hispanic, 16% Black, 27% diabetes, median platelet count 98K/mm^3 (IQR 73–140), median albumin 3.6 g/dL (IQR 3.1–4), median MELD 9 (IQR 8–11), 25% with ascites, 23% with hepatic encephalopathy, 15% with varices, 56% treatment experienced, 62% genotype 1a (44% Q80K positive [n = 25]). CP-B/C vs CP-A had more early treatment discontinuations (11% vs 1%), adverse events (AEs) requiring hospitalization (22% vs 2%), infections receiving parenteral antibiotics (20% vs 1%) and decompensating events (20% vs 3%) (all $p < 0.01$). There were 2 deaths: 1 CP-B/C (liver-related) and 1 CP-A (not liver-related). Among eligible patients (n = 104), SVR12 was achieved by 66% of CP-B/C vs 93% of CP-A ($p < 0.01$). In multivariate analysis, CP-A independently predicted SVR12 (OR 7.68, 95% CI 2.13–27.7). Compared to PI-triple therapy, SIM+SOF±RBV patients more frequently achieved SVR12 with fewer early discontinuations, AEs requiring hospitalization, infections requiring parenteral antibiotics and decompensation (Figure). Safety outcomes occurred at similar frequency to matched untreated controls (Figure).

Conclusions: CP-B/C treated with SIM+SOF±RBV for 12 weeks have significantly more adverse events and reduced SVR rates compared to CP-A. However, SIM+SOF±RBV are both safer and more efficacious than prior PI-triple therapy. Frequency of AEs, infections and decompensation were similar to matched untreated controls suggesting safety outcomes seen with SIM+SOF±RBV therapy is reflective of the natural history of cirrhosis and are not drug-specific.

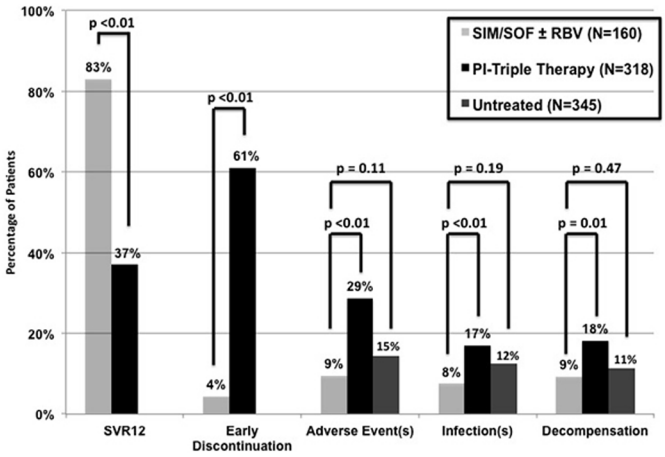

Figure: Virologic and safety outcomes, SIM/SOF±RBV vs. matched PI-triple therapy controls and matched untreated controls.

P0877

HCV RNA LEVELS ASSESSED AT EARLY STAGES OF SOFOSBUVIR/RIBAVIRIN DUAL THERAPY IDENTIFY RELAPSE PATIENTS

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Background and Aims: Current EASL guidelines recommend HCV RNA measurements at specific time points of sofosbuvir (SOF) therapy to ensure treatment efficacy. However, it is not well studied, how HCV RNA results during SOF-including regimens have to be interpreted.

We here aimed to analyse whether HCV RNA levels during different approved SOF-based therapies predict the final treatment outcome comparing the COBAS TaqMan (CTM) and the Abbott RealTime (ART) assay.

Methods: Samples were collected from consecutive HCV-monoinfected patients (genotype 1–5) at weeks (w) 0, 1, 2, 4, 8, 12, 16, 20 and 24 of SOF-based treatments at two German University clinics (Frankfurt, Hanover) and tested for quantitative HCV RNA by CTM and ART. 171 patients with at least 4w follow up post treatment were included. 87 patients were treated with SOF/ribavirin (RBV), 43 with SOF/RBV and pegylated-interferon alfa (PEG-IFN), 39 with SOF and a second direct acting antiviral (DAA), either simeprevir (n = 35) or daclatasvir +/− RBV (n = 5).

Results: All SOF/DAA, 95% of SOF/PEG-IFN/RBV and 98% of SOF/RBV treated patients were HCV RNA negative with both assays at the end of therapy. Relapse occurred in 10%, 5% and 33%, respectively. HCV RNA levels during the first four weeks of SOF/DAA and SOF/PEG-IFN/RBV treatment did not differ between relapse and SVR patients. In contrast, during SOF/RBV treatment HCV RNA levels were significantly higher in relapse patients according to both CTM and ART at w1 ($p < 0.0001$; and $p = 0.0005$), w2 ($p < 0.0001$ and $p < 0.0001$) and w4 ($p = 0.0002$ and $p = 0.0006$). Patients with an HCV RNA level of >40 IU/ml by CTM or >20 IU/ml by ART at w2 had a significantly lower chance to achieve SVR (28% vs. 92%, $p < 0.0001$ and 43% vs. 96%, $p < 0.0001$, respectively). Sensitivity to detect relapse patients was 85% and 96%, respectively.

By w8 the far majority of SOF/DAA treated patients was HCV RNA undetectable by CTM (97%), while almost half of the patients were detectable by ART (48%). Detectable patients had a lower but still reasonable SVR rate (70% vs. 100%, $p = 0.09$). All three patients with a detectable HCV RNA at w8 of SOF/PEG-IFN/RBV therapy (all by ART) and even one tested positive at the end of SOF/PEG-IFN/RBV therapy achieved SVR.

Conclusions: The predictive value of on-treatment HCV RNA levels seems to be limited for highly potent regimens including SOF and either a second DAA or PEG-IFN. In contrast, early on-treatment HCV RNA levels are closely associated with the outcome of SOF/RBV therapy.

P0878

FIRST RIBAVIRIN-FREE SOFOSBUVIR AND SIMEPREVIR TREATMENT OF HEPATITIS C GENOTYPE 1 PATIENTS WITH SEVERE RENAL IMPAIRMENT (GFR <30 mL/min OR DIALYSIS)

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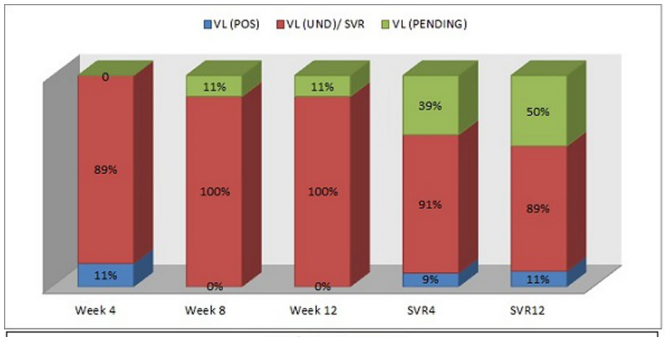
Background and Aims: Hepatitis C (HCV) prevalence in hemodialysis (HD) patients is approximately 13.5% which is significantly higher than the prevalence in general population. However, there is limited data on the use of direct acting antivirals (DAA) or ribavirin-free regimen in this cohort. Our aim is to present the preliminary results of the first ribavirin-free regimen (sofosbuvir and simeprevir) to treat HCV genotype 1 (GT) patients with severe renal impairment (GFR <30 ml/min or HD).

Methods: Eighteen HCV patients (11 GT1a, 7 GT1b) with severe renal impairment (15 on HD, 3 with mean GFR of 16 ml/min) underwent open label treatment with sofosbuvir and simeprevir. Sixteen patients had advanced fibrosis (>F2) and 10 patients had cirrhosis (56%). All patients received full dose simeprevir of 150 mg daily. Sofosbuvir dose was reduced to 200 mg daily in 15 patients and 400 mg every other day in 3 patients. The length of therapy was 12 weeks in 17 patients. Treatment duration was extended to 24 weeks in 1 cirrhotic patient who had low level viremia at week

4. Sixteen patients had completed treatment and 2 others are still undergoing treatment at their 7th and 16th weeks.

Results: All patients had excellent on-treatment response and became aviremic by 8 weeks (2 patients had low level viremia at week 4). One patient developed new onset hepatic encephalopathy and another developed uncontrolled diarrhea, both requiring hospitalizations during treatment. Minor AE's fatigue (28%), anemia (11%), rash/itching (11%) and nausea (5%) were managed medically and there were no treatment discontinuations. Of the 16 patients who completed treatment, only 9 patients reached relevant milestones. As per the current per-protocol analysis, SVR4 was seen in 91% and SVR12 in 89%. One cirrhotic patient (prior PI failure) relapsed within 4 weeks after completion of the treatment. We are still following the response in 9 other patients which will be presented at the time of EASL meeting.

Conclusions: Simeprevir and reduced dose sofosbuvir is safe, well tolerated and efficacious in HCV GT 1 patients with preliminary SVR4 of 91% and SVR12 of 89%. Our data represents the first ribavirin-free DAA regimen in this difficult to treat cohort of HCV with severe renal impairment or HD.



Virologic Response						
	Week 4 N=18	*Week 8 N=17*	*Week 12 N=17*	*Week 24 N=1*	*SVR 4 N=11*	*SVR 12 N=9*
Undetectable, n (%)	16 (89%)	17 (100%)	17 (100%)	0 (0)	10 (91%)	8 (89%)
Detected, n (%)	2 (11%)	0 (0)	0 (0)	0 (0)	1 (9%)	1 (11%)
Pending, n (%)	0 (0)	1 (6%)	1 (6%)	1 (6%)	7 (39%)	9 (50%)

P0879

EXAMINING THE CLINICAL COURSE OF GENOTYPE 1 CHRONIC HEPATITIS C PATIENTS TREATED WITH THE COSMOS REGIMEN: INCLUDING PATIENTS WITH ADVANCED LIVER DISEASE, AND EAST ASIAN ANCESTRY

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Background and Aims: The COMSOS phase 2 trial showed high cure rates and a favorable side effect profile of a 12-wk regimen of Sofosbuvir (SOF) and Simeprevir (SIM) in patients with a genotype 1 Hepatitis C infection. However, given the small number of patients in the COSMOS trial, there is uncertainty regarding the efficacy and safety of this combination therapy. We now report our experience with the COSMOS regimen in the multiethnic population of Hawaii, including patients of East Asian ancestry and decompensated cirrhosis.

Methods: Retrospective review of 99 patients treated with a fixed dose regimen of SIM 150 mg and SOF 400 mg daily, beginning January 2014 at a single referral center. We collected data on demographics, side effects, laboratory studies and SVR (sustained virological response). Statistical analysis was performed with Stata v8.2 software.

Results: 99 patients began treatment prior to October 2014. Baseline characteristics: 63.3% cirrhotic (18.2% of those Child–Pugh Class B/C), 38% Asian, 12% Pacific Islander, 64% male, mean age 61.5±8.4, mean BMI 27±5.9, 62% diabetic, 62% genotype 1a, 22/42 IL28B non-CC genotype, 7/32 positive for Q80K mutation.
At interim analysis, 72 patients have reached week 4 and 45 have reached week 12 post-treatment. Overall, the SVR12 rate is 86.5%. 100% decompensated cirrhotic patients achieved SVR12, compared to 85.2% of cirrhotic patients and 82.1% of non-cirrhotic patients. 90% of Asian patients reached SVR12 compared to 84% of non-Asians. Treatment naive patients had higher SVR12 rates than treatment experienced patients (96% vs 82%). Main side effects: headache 16.2%, fatigue 24.2%, pruritis 14.1%; none were >grade 2 in severity. There were no differences in side effect profiles of patients with decompensated cirrhosis. Pruritis was the only statistically significant difference between Asians and non-Asians (24% vs 8%).

Conclusions: The 12 week fixed dose course of SIM+SOF was well tolerated in a multiethnic population of primarily cirrhotic patients, including those with decompensated disease, with the SVR12 rates at interim analysis comparable to COSMOS data. There was a trend toward better SVR12 rates in patients with decompensated cirrhosis and of Asian ancestry possibly due to higher Simeprevir exposures. Higher incidence of adverse side effects was not observed with an exception of higher rate of pruritis in Asians. Complete data on SVR4 and SVR12 will be available on all patients by April 2015.

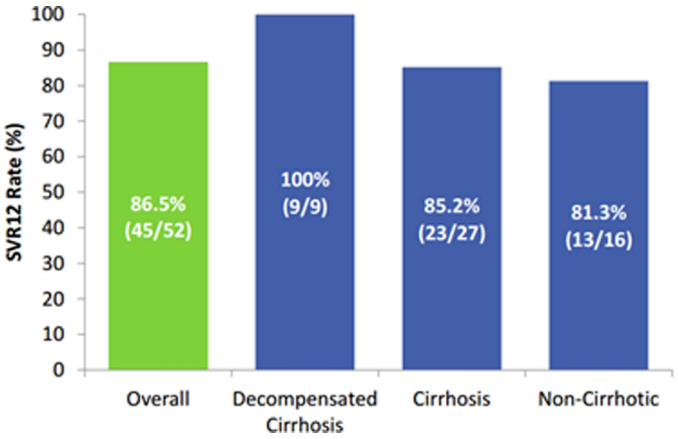

Figure 1. SVR12 rates according to levels of fibrosis.

P0880

PHARMACOGENOMIC STUDY TO PREDICT RIBAVIRIN AND PROTEASE-INHIBITOR-RELATED ANEMIA IN HEPATITIS C

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Background and Aims: Anemia is a frequent adverse effect, associated with ribavirin (RBV), in patients with hepatitis C (HCV). It has become the main limitation, in terms of safety, of the addition of first-generation protease inhibitors. We aimed to assess the impact of genes ITPA and SLC28A3 on HCV patients receiving peginterferon (PEG-IFN) + RBV + Boceprevir or Telaprevir.

Methods: Multicenter study including 161 patients with HCV genotype 1. Patients were divided according to treatment: (a) Telaprevir 750 mg/8 h for 12 weeks + PEG-IFN + RBV 48 weeks (n = 95); (b) Boceprevir 800 mg/8 h for 44 weeks + PEG-IFN + RBV 48 weeks (including lead-in phase) (n = 66). We genotyped: (a) ITPA (rs1127354, rs6051702, rs7270101); (b) SLC28A3 (rs56350726, rs10868138, rs11854484). We defined clinically

significant anemia when, at least, one of the following aspects happened: (a) hemoglobin <8.5 g/dL during treatment; (b) blood transfusion requirement; (c) erythropoietin use.
Results: Clinically significant anemia occurred in 43.9% (69/157) of patients, regardless of Boceprevir (37.7%; 20/53) or Telaprevir use (47.1%; 49/104) (p = 0.263). RBV dose was reduced in 62.1% (100/161) of patients, erythropoietin was used in 34.8% (55/158) and 16.4% (26/159) required blood transfusion. SLC28A3 rs11854484 was associated with clinically significant anemia [CC/CT genotypes 33.3% (26/78) *vs.* TT genotype 56.3% (36/64) (p = 0.006)]. Furthermore, it was related to the needed of transfusion [CC 0% (0/18) *vs.* CT 13.1% (8/61) *vs.* TT 26.6% (17/64), p = 0.016]. Similarly, ITPA rs1127354 was linked to clinically significant anemia [AA/AC genotypes 18.8% (3/16) *vs.* CC genotype 45.2% (61/135), p = 0.043]. In multivariate analysis, SLC28A3 rs11854484 TT genotype (OR 2.25, 95% CI 1.03–4.90; p = 0.042), female sex (OR 2.97, 95% CI 1.27–6.94; p = 0.012) and Hb drop at week 4 (OR 1.48, 95% CI 1.19–1.85; p = 0.0001) were independently associated with clinically significant anemia.
Conclusions: In patients receiving first generation protease inhibitors, SLC28A3 rs11854484 predicted clinically significant anemia. Additionally, SLC28A3 rs11854484 genotype CC protected from blood transfusion, identifying a subgroup of patients with better tolerance to triple therapy. The impact of these SNPs on RBV-based IFN-free regimen requires further studies.

P0881
REAL WORLD EFFECTIVENESS AND COST OF SIMEPREVIR- AND/OR SOFOSBUVIR-BASED HCV TREATMENTS: $175,000 PER SVR12

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Background and Aims: Simeprevir- (SMV) and/or sofosbuvir- (SOF) based regimens were effective in clinical trials, but data on their performance in real world clinical practice are needed.
Methods: Data were collected on the complete cohort of patients with chronic HCV infection who started treatment at the Mount Sinai Medical Center (MSMC) after SMV and SOF received FDA approval (12/2013–6/2014). Patients with HIV infection or liver transplantation were excluded. Data on 223 patients treated with telaprevir (TVR)/boceprevir (BOC) were included for comparison (Bichoupan et al., Hepatology, 2014). Advanced fibrosis/cirrhosis was defined as a FIB-4 score ≥3.25. Sustained virologic response-12 (SVR12) was defined as an undetectable HCV RNA 12 weeks after the end-of-treatment (EOT). Costs of treatment included laboratory tests, physician fees, adverse event management and pharmaceuticals, which were obtained from the Red Book Wholesale Acquisition Cost database.
Results: The SMV/SOF era cohort included 514 patients on regimens that contained SMV and/or SOF. The median age was 60 yr [interquartile range (IQR): 54–64 yr], 54% had a FIB-4 score ≥3.25, 14% were black, and 40% were previously untreated. In comparison to the TVR/BOC cohort, the SMV/SOF cohort was significantly older (59 vs. 55 yr, p < 0.01), had lower albumin (3.90 g/dL vs. 4.15 g/dL, p < 0.01), and had a higher percentage with FIB-4 ≥3.25 (54% vs. 44%, p = 0.02). In the SMV/SOF era cohort, 224 had sufficient follow up to evaluate SVR12: 210 (94%) completed therapy, 50 (22%) relapsed, 10 (5%) never became HCV viral load undetectable, and 2 (1%) died. Relapse after SVR4 was confirmed in 5 (3%). SVR rates for specific regimens are presented in Table 1. Among patients on SMV/SOF +/− RBV, those who failed on a prior protease-inhibitor had a lower SVR12 rate than other patients: 25/35 (71%) vs. 57/62 (92%), p = 0.02. The cost of care for the 224 patients was $28.3 million. Pharmaceuticals accounted for 97% of costs. Patients who failed therapy accounted for 30% of costs. The mean cost per SVR12 was about $175,000, which is lower than $178,000 for TVR-based treatment, but not significantly different (p = 0.62).

Table 1. Cost per SVR by drug regimen and genotype

Regimen (duration)	N (column%)	Total cost	SVR12 rate (%)	Cost per SVR12 (SD)
Total	224	$28,300,000	162/224 (72%)	$175,000 ($51,500)
SOF/RBV (12/24 weeks)	72 (32%)	$8,350,000	40/72 (56%)	$209,000 ($86,000)
Genotype 1 (24 weeks)	22 (30%)	$3,280,000	4/22 (18%)	$821,000 ($260,000)
Genotype 2 (12/16 weeks)	41 (57%)	$3,800,000	34/41 (83%)	$112,000 ($29,000)
Genotype 3 (24 weeks)	7 (10%)	$849,000	1/7 (14%)	$849,000 ($468,000)
Genotype 4 (24 weeks)	2 (3%)	$413,000	1/2 (50%)	$413,000 ($96,000)
SOF/PEG/RBV (12 weeks)	55 (25%)	$5,180,000	40/55 (73%)	$129,000 ($16,000)
Genotype 1 (12 weeks)	44 (80%)	$4,120,000	31/44 (70%)	$133,000 ($18,000)
Genotype 4 (12 weeks)	11 (20%)	$1,060,000	9/11 (82%)	$117,000 ($2,000)
SOF/SMV (12 weeks)	27 (12%)[a]	$4,140,000	22/27 (81%)	$188,000 ($4,450)
SOF/SMV/RBV (12 weeks)	70 (31%)	$10,700,000	60/70 (86%)	$178,000 ($14,000)

[a] One patient who received SOF/SMV was infected with genotype 4 HCV.

Conclusions: Patients treated with SMV and/or SOF regimens were older and had more advanced liver disease than those in the TVR/BOC cohort. Importantly, the overall SVR12 rate increased significantly while cost remained steady. Aging of the HCV population and worsening liver disease may contribute to reduced efficacy compared to clinical trials.

P0882
PILOT STUDY OF EZETIMIBE FOR CHRONIC HEPATITIS C INFECTION: EFFECT OF TREATMENT IN PLASMA, BILE AND STOOL VIRAL LOAD IN HUMANS

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Background and Aims: Even though direct acting antivirals (DAAs) are showing impressive efficacy and safety in the treatment of hepatitis C virus (HCV) infection, the potential for resistance and high prices may still limit their broad use. Hence, it is of interest to continue exploring alternative host targeted therapies that could be used as complements to current DAAs. A recently report showed that Niemann-Pick C1-Like 1 (NPC1L1) protein acts as a post binding HCV entry factor. Based on this observation in this study we evaluated the impact of ezetimibe treatment, an approved and safe NPC1L1 antagonist, on HCV viral load in plasma, bile and stools.
Methods: Patients with chronic HCV infection were studied after obtaining informed consent. Exclusion criterial included presence of gallstones or history of cholecystectomy, treatment with lipid-lowering therapy and antiviral therapy. Ezetimibe was administered in a dose of 10 mg two times a day for 12 weeks, along with a standardized diet. Patients underwent an upper endoscopy for obtaining bile samples at baseline and at weeks 4 and 12. Stool specimens were collected and preserved in RNALater™. Contamination of stools with occult blood was studied using an immunological human haemoglobin assay. PowerMicrobiome™ RNA Isolation Kit was used for RNA extraction from both bile and stools. HCV RNA level was measured in plasma, bile and stools with Roche COBAS Taqman v2™.
Results: Ten patients, with a mean age 63 years, predominantly genotype 1b and 50% cirrhotic, were recruited. At baseline, HCV RNA was detected in bile in 8 patients, with a median of 58 IU/mL, 4 logs lower than plasma viral load (median 2,044,500 IU/mL). In stools,

HCV RNA was detectable in 8 patients with a median of 125 IU/mL. Despite having a significant decrease in cholesterol level from 143 to 123 mg/dL (p = 0.04) after 12 weeks of treatment, plasma viral load decreased only 0.3 log (p = 0.2) in plasma. Similarly, non-significant decreases in bile and stool viral load were found (see table).

	Baseline	12 weeks	p
Age, years, mean±SD	62.6±7.7	–	–
Male gender, n (%)	5 (50%)	–	–
Cirrhosis, n (%)	5 (50%)	–	–
HCV genotype, n 1a / 1b / 3a	1 / 8 / 1	–	–
Body weight, kg, mean±SD	66.97±15.3	67.26±15.2	0.39
BMI, kg/m^2, mean±SD	24.52±2.69	24.62±2.53	0.41
ALT, U/mL, mean±SD	86±68	86±56	0.99
AST, U/mL, mean±SD	78±67	79±58	0.89
Bilirubin, mg/dL, mean±SD	1.0±0.75	1.1±0.8	0.70
Glucose, mg/dL, mean±SD	99±19	105±49	0.58
Plasma total cholesterol, mg/dL, mean±SD	143±36	123±30	0.04*
Plasma LDL cholesterol, mg/dL, mean±SD	64±25	51±15	0.04*
Plasma HDL cholesterol, mg/dL, mean±SD	54±15	56±22	0.59
Plasma triglycerides, mg/dL, mean±SD	86±44	84±44	0.82
Plasma viral load, mean log IU/mL ±SD	5.93±1.39	5.65±1.31	0.23
Bile viral load, mean log IU/mL ±SD	1.84±1.81	1.16±1.10	0.12
Stool viral load, mean log IU/g ±SD	1.71±1.02	1.65±0.99	0.87

Conclusions: Our results show that HCV RNA can be found and measured in bile and stools of infected patients. No significant changes in plasma, bile or stool viral load were demonstrated after ezetimibe treatment. The lack of response in humans, despite encouraging in vitro data, may be explained by a low expression level of NPC1L1 in the liver compared to the gut or by the lower exposure of liver NPC1L1 to ezetimibe *in vivo*.

P0883

TREATMENT FOR HEPATITIS C VIRUS INFECTION AMONG PEOPLE WHO INJECT DRUGS IN THE OPIOID SUBSTITUTION SETTING: THE ETHOS STUDY

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Background and Aims: Assessment and treatment for hepatitis C virus (HCV) among people who inject drugs (PWID) is low and strategies are needed to enhance access to care. This study aims to evaluate the effectiveness of HCV treatment among PWID.
Methods: Enhancing Treatment for Hepatitis C in Opioid Substitution Settings (ETHOS) is a prospective observational cohort, evaluating a model for the provision of HCV assessment and treatment among people with a history of injecting drug use and chronic HCV. Recruitment occurred through six opioid substitution treatment (OST) clinics, two community health centres and one Aboriginal community controlled health organisation in NSW, Australia. Participants initiating pegylated interferon/ribavirin (PEG-IFN/RBV) treatment between February 2009 and December 2012 (genotype 1, G1) or June 2013 (genotypes 2 and 3, G2/3) were included, to allow for adequate post-treatment follow-up. Statistical analyses were performed using Chi-squared or Fisher's exact tests, as appropriate.
Results: Among 415 participants, 27% (n = 111) commenced treatment. Among those treated between 2009 and 2013 (n = 104, mean age 43 years, 77% male), 36% (n = 37) had injected drugs in the past six months and 62% (n = 64) were currently receiving OST. In an intent-to-treat analysis, the sustained virological response (SVR) was 65% overall (68 of 104), 69% in G1 (20 of 29) and 64% in G2/3 (48 of 75). There was no difference in SVR between those never (74%, 23 of 31) and currently (66%, 42 of 64, P = 0.484) receiving OST. SVR was similar among those who had injecting drugs in the past six months (70%, 26/37) compared to those who had not (64%, 43/67, P = 0.665).
Conclusions: Response to treatment in this population was high and active injecting drug use did not compromise treatment response. This data suggests that targeted initiatives to enhance HCV treatment in OST or community health clinics can be successful.

P0884

INTERFERON AND RIBAVIRIN-FREE THERAPY WITH SOFOSBUVIR AND DACLATASVIR IN A REAL-LIFE COHORT OF DIFFICULT-TO-TREAT HIV/HCV-COINFECTED PATIENTS WITH AND WITHOUT PORTAL HYPERTENSION

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Background and Aims: Despite promising results for the combination of sofosbuvir and daclatasvir (SOF/DCV) in patients with HCV-monoinfection, there are currently no reports on its use in HIV/HCV-coinfected patients (HIV/HCV). The aim of our study was to investigate the use of SOF/DCV in HIV/HCV, who have an urgent need for effective treatment options.
Methods: All patients were treated with once-daily SOF (400 mg) and DAC. The dose of DAC was adjusted to the cART regimen, as recommended by the European label. The following treatment durations will be applied: HCV-genotype (HCV-GT) 1/4 with previous HCV protease inhibitor (PI) failure or cirrhosis: 24 weeks; HCV-GT3: 24 weeks; all other patients: 12 weeks. HCV-RNA was assessed using the Abbott RealTime HCV assay with a lower limit of quantification (LLOQ) and detection of 12 IU/mL.
Results: All of the 15 patients who have started SOF/DAC were on cART. Six (40%) patients were treatment experienced, including 2 (13%) patients with previous PI failure. The majority of patients had HCV-GT1a (10 [67%]), while HCV-GT3 and 4 were observed in 4 (27%) patients and 1 (7%) patient, respectively. Eleven (73%) patients had high baseline HCV-RNA levels (>600,000 IU/mL) and 14 (93%) patients had the IL28B non-C/C genotype. All patients had either F3 fibrosis (5 [33%]), cirrhosis (7 [47%]), or an extrahepatic manifestation of CHC (3 [20%]). These were cryoglobulinemia with leg ulcers, cryoglobulinemia with glomerulonephritis and end stage renal disease, as well as primary central nervous system lymphoma. The median liver stiffness was 10.2(12.7) kPa and the mean hepatic venous pressure gradient (HVPG) among patients with advanced fibrosis was 9.1±4.5 mmHg. Portal hypertension (HVPG ≥6 mmHg) and clinically significant portal hypertension (CSPH; HVPG ≥10 mmHg) were observed in 9 (60%) and 5 (33%) patients, respectively. At week 4, 1/5 patients had HCV-RNA <LLOQ, while at week 8, 2/5 patients had HCV-RNA <LLOQ and 1/5 patients achieved undetectable HCV-RNA.
The results will be updated prior to the presentation at the meeting to include more than 30 difficult-to-treat HIV/HCV as well as preliminary sustained virologic response rates.
Conclusions: The combination of SOF/DCV allows for the IFN and RBV-free treatment of nearly all HIV/HCV, including patients with cirrhosis and CSPH or extrahepatic manifestations. Results from

our thoroughly documented real-life cohort will provide important evidence for the use of SOF/DCV in this special population.

P0885

MOST PATIENTS OF HEPATITIS C VIRUS INFECTION IN INDIA PRESENT LATE FOR ANTIVIRAL TREATMENT: AN EPIDEMIOLOGICAL STUDY FROM A NORTH INDIAN CENTER

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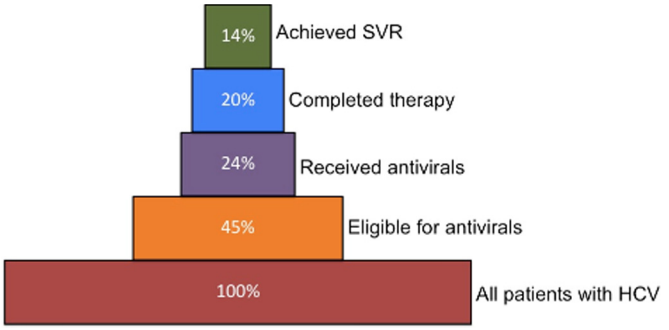
Background and Aims: Antiviral therapy with Peg-interferon and Ribavirin is offered only to those patients of HCV who are in stage of chronic hepatitis or early cirrhosis. Patients of advanced liver disease do not tolerate this therapy. Since HCV is asymptomatic in early stages and usually presents with advanced disease, the eligibility for antiviral therapy is thus limited. There are no studies from India, which look into clinical spectrum of HCV infection at presentation, with reference to the eligibility and success of antiviral treatment. Our aim is to study the spectrum of presentation of HCV infection, determine their eligibility for antiviral treatment, and follow those treated for treatment response.
Methods: The records of all consecutive patients of HCV, >14 years age, who presented to our department between 2010 and 2014, were analyzed for categorization into chronic hepatitis, cirrhosis and hepatocellular carcinoma; and to assess eligibility for antiviral treatment. Patients with detectable HCV RNA who have chronic hepatitis or Child A cirrhosis were considered eligible for antiviral treatment with Peg-Interferon and Ribavirin. Patients who received treatment were followed for sustained viral response.
Results: A total of 777 patients (median age 49 [range 15–95] years, males 69%) presented during the study period with HCV. Cirrhosis was the most common presentation (56%) followed by chronic hepatitis (37%) and HCC (7%). Of patients who had cirrhosis (including those with HCC) 36% had Child A cirrhosis; 51% had Child B cirrhosis and 14% had Child C cirrhosis. Out of all the 777 HCV patients only 347 (45%) were eligible for antiviral treatment. Among the remaining 430 patients, in 326 (76%) the disease was far too advanced to offer any antiviral treatment. Of patients eligible for antiviral treatment only 54% (189/347) actually received antiviral treatment and 81% (153/189) patients could complete the antiviral course. Of them 70% (107/153) only could achieve the SVR (Figure 1).
Conclusions: Most patients of HCV infection in India present late and only about 45% are eligible for antiviral treatment with Peg-interferon and Ribavirin. At presentation 56% patients already have cirrhosis and 7% have HCC. Since HCV is usually asymptomatic at treatable stage, awareness about screening should be increased so that more patients are diagnosed at treatable stage.



P0886

EFFICACY AND SAFETY OF GRAZOPREVIR/ELBASVIR +/− RBV FOR 12 WEEKS IN PATIENTS WITH HCV G1 OR G4 INFECTION WHO PREVIOUSLY FAILED PEGINTERFERON/RBV: C-EDGE TREATMENT-EXPERIENCED TRIAL

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Background and Aims: Efficacious, well-tolerated, interferon/ribavirin (RBV)-free, short-duration regimens are desirable for HCV-infected patients who failed prior therapy. In the Phase 2 C-WORTHY study, 12- or 18-weeks' dosing of once daily grazoprevir (GZR) 100 mg + elbasvir (EBR) 50 mg, ± twice-daily RBV, resulted in SVR12 in >90% of cirrhotic HCV genotype (GT) 1-infected patients with prior null response to peginterferon/RBV (PR). The Phase 3 C-EDGE Treatment-Experienced (TE) study is evaluating the efficacy and safety of once-daily, GZR/EBR 100 mg/50 mg fixed-dose combination (FDC) tablet, ± twice-daily RBV, for 12 or 16 weeks in cirrhotic or non-cirrhotic HCV GT1-, 4-, or 6-infected patients who have failed prior PR therapy.
Methods: This trial enrolled cirrhotic and non-cirrhotic HCV GT1-, 4-, or 6-infected patients who failed prior PR therapy. HIV co-infected patients were not excluded. Patients were randomized in a 1:1:1:1 ratio to receive GZR/EBR QD for 12 or 16 weeks, ± twice-daily RBV. Arms were stratified for cirrhosis status and prior PR treatment response. HCV RNA was assessed using COBAS TaqMan v2.0 (LLoQ 15 IU/mL). The primary endpoint was HCV RNA <15 IU/mL 12 weeks after end of treatment (SVR12).

Table: SVR4 by patient characteristics

	GZR/EBR for 12 weeks (N = 105)	GZR/EBR + RBV for 12 weeks (N = 104)
SVR4, n/N (%)		
Overall	100/105 (95%)	102/104 (98%)
GT1a	55/58 (95%)	54/56 (96%)
GT1b (or other than GT1a)	38/38 (100%)	33/33 (100%)
GT4	7 [a]/9 (78%)	15/15 (100%)
Null responder	42/45 (93%)	42/44 (95%)
Partial responder or Relapse	59/60 (98%)	60/60 (100%)
HIV/HCV	6/6 (100%)	5/5 (100%)
Cirrhosis	34/37 (92%)	35/36 (97%)
Virologic failure		
Breakthrough	0	0
Relapse	4	2
Discontinued		
Total	2 [a,b]	0
Of which due to AE	1	0

[a] Death – Cirrhotic, GT4-infected subject died of lymphoma.
[b] Discontinued study med at week 6 due to mood lability: achieved SVR4.

Results: This study is fully enrolled; complete SVR12 data from the 12 week arms will be available at the ILC meeting. Of 209 patients enrolled in the 12-week arms; 66% were male, 34% were cirrhotic, 5% were HIV co-infected, 23% were Black, and 9% were Hispanic. No GT6-infected patient were randomized to 12 weeks treatment. Mean viral load at baseline was 6.3log10 IU/mL. Previous response to PR: relapse in 35%, partial response in 22% or null response in 43%. All patients in 12-week arms have completed 4

weeks of follow-up. Common adverse events during treatment were fatigue (19%), headache (15%), and nausea (9%). The table displays preliminary SVR4 results.

Conclusions: This is an ongoing, Phase III study among subjects with HCV GT1 or 4 infection who failed prior PR therapy, including large numbers of Black/African-American and cirrhotic patients. Preliminary results showed a 12 week regimen of GZR/EBR FDC was highly efficacious with respect to SVR4 among these hard-to-cure patients, and had a favorable safety profile.

P0887

C-EDGE CO-INFECTED: PHASE 3 STUDY OF GRAZOPREVIR/ELBASVIR IN PATIENTS WITH HCV/HIV

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Background and Aims: The combination of grazoprevir (GZR, MK-5172, a HCV NS3/4 protease inhibitor) and elbasvir (EBR, MK-8742, an HCV NS5A inhibitor), an interferon-free, ribavirin-free, once-daily, fixed-dose combination (FDC) tablet has demonstrated robust efficacy and an excellent safety profile in diverse populations. C-EDGE HIV/HCV is an on-going, phase III study to assess the safety and efficacy of GZR/EBR FDC in HIV co-infected patients with chronic HCV genotype (GT) 1, 4 or 6 infection, with or without cirrhosis.

Methods: Subjects were eligible for enrollment if they met all of the following criteria: chronic HCV GT 1, 4 or 6 infection, cirrhotic or non-cirrhotic, no prior HCV treatment, HIV infection on a stable antiretroviral (ARV) regimen with a CD4 >200 cells/mm^3 and an HIV RNA <20 copies/mL, or HIV-treatment naive with CD4 >500 cells/mm^3 and VL <50,000 copies/mL. ARV therapy included a dual NRTI of tenofovir or abacavir, and lamivudine or emtricitabine; and either raltegravir, dolutegravir or rilpivirine. Presence of cirrhosis was defined by either (1) histology, (2) fibroscan >12.5 kPa, or (3) fibrotest >0.75 and APRI >2.0. All patients received open-label GZR/EBR 100 mg/50 mg FDC QD for 12 weeks. The primary endpoints were sustained virologic response (SVR) at follow-up week 12 (COBAS TaqMan v2.0 [lower limit of quantitation <15 IU/mL]), and assessment of safety and tolerability.

Results: 262 subjects were screened and 218 subjects met criteria for enrollment. Table 1 displays baseline characteristics for the enrolled subjects. In preliminary results among 214 subjects for whom HCV RNA data was available, 212 (97%) achieved SVR4. Virologic failure occurred in 2/218 (0.9%); both categorized as relapses at FW4. Overall adverse events (AEs) were reported in 157/218 subjects (72%); drug-related adverse events occurred in 72/218 subjects (33%); serious AEs occurred in 2/218 subjects (0.9%). The most common AEs reported included fatigue (15%), headache (15%), nausea (10%), diarrhea (10%), and insomnia (9%). Through FW4, no patient has discontinued due to AEs or laboratory abnormalities. Two patients experienced transient HIV viremia (HIV RNA >200 copies/mL); both patients were subsequently undetectable.

Conclusions: This is a large, ongoing, Phase III study among subjects co-infected with HIV and HCV GT1, 4 or 6 including significant numbers of Black/African-American and cirrhotic patients. A 12 week regimen of GZR/EBR FDC was highly efficacious with respect to SVR4 among cirrhotic and non-cirrhotic HIV co-infected subjects with HCV GT 1, 4 or 6 infection, and has a favorable safety profile. SVR12 results will be presented at the ILC meeting.

	All patients (N = 218)
Age, years, mean	49
Male, %	83.9
Race, %	
White	76.6
Black or African-American	17.4
Baseline HCV RNA, IU/mL, mean	6.03 $\log_{10}$
Baseline CD4 count, cells/μL, median (IQR)	568 (424–766)
HCV Genotype, %	
1a	65.6
1b	19.3
1 non-subtypeable	1.8
4	12.8
6	0.5
Cirrhotic, %	16.1
Antiretroviral therapy, %	
Abacavir containing regimen	17.1
Tenofovir containing regimen	82.9
Raltegravir	52.9
Dolutegravir	29.9
Rilpivirine	17.1

P1353

A SINGLE TABLET REGIMEN OF LEDIPASVIR/SOFOSBUVIR FOR 12 WEEKS IN HCV GENOTYPE 1 OR 4 INFECTED PATIENTS WITH HIV-1 CO-INFECTION: THE PHASE 3 ION-4 STUDY

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Background and Aims: Historically HIV co-infection was considered a negative predictor of HCV response to treatment with interferon/ribavirin (IFN/RBV). For sofosbuvir-based regimens, HIV/HCV patients have achieved similar sustained virologic response (SVR) rates as HCV monoinfected patients. We evaluated the safety and efficacy of the IFN-free, RBV-free, single tablet regimen of ledipasvir/sofosbuvir (LDV/SOF) in HCV genotype 1 or 4 patients co-infected with HIV-1 in the Phase 3 ION-4 study.

Methods: HCV treatment naïve and experienced HIV co-infected patients on stable, approved antiretroviral (ARV) regimens were enrolled and received LDV/SOF (90 mg/400 mg) once daily for 12 weeks. Patients with compensated cirrhosis were eligible. Permitted concomitant ARVs included tenofovir/emtricitabine (TDF/FTC) with raltegravir (RAL), efavirenz (EFV) or ripilvirine (RPV). Safety evaluations included adverse event (AE) and standard laboratory parameter monitoring in addition to enhanced renal toxicity monitoring, CD4 count and HIV-1 RNA levels. The primary endpoint was SVR12.

Results: 335 patients with GT1a (75%), GT1b (23%) and GT4 (2%) were enrolled in the study; 83% were male, 61% were white, mean age was 52 years (range 26–72), mean baseline HCV RNA was 6.7 $\log_{10}$ IU/mL (range 4.1–7.8), median baseline CD4 count was 662 cells/uL (Q1, Q3 = 469, 823), 20% had cirrhosis, 24% were *IL28B* CC genotype and 55% had not responded to prior HCV treatment. Most patients were taking EFV (48%) or RAL (44%). The table shows SVR4 by ARV regimen. Overall, SVR4 was 96% (322/335); 2 patients had on-treatment virologic failure due to non-compliance and 8 had virologic relapse after discontinuing treatment. Overall, SVR4 (97%) among non-cirrhotic (F0–F3) patients was similar to SVR4 (94%) among cirrhotic (F4) patients. No patient had confirmed HIV virologic rebound (HIV-1 RNA ≥400 copies/mL). No patients

discontinued study drug due to an AE. AEs occurring in ≥10% of patients were headache (25%), fatigue (22%) and diarrhea (11%). No significant lab abnormalities were observed.

Table: SVR4 by ARV regimen and overall

Virologic response	TDF/FTC+EFV (N = 160)	TDF/FTC+RAL (N = 146)	TDF/FTC+RPV (N = 29)	Overall (N = 335)
SVR4, n (%)	152 (95)	142 (97)	28 (97)	322 (96)
On-treatment failure, n (%)	1 (<1)	0	1 (3)	2 (<1)
Relapse, n (%)	7 (4)	1 (<1)	0	8 (2)
Other, n (%)	0	3 (2)	0	3 (<1)

Conclusions: The IFN-free, RBV-free, single tablet regimen of LDV/SOF administered once daily for 12 weeks is highly effective and well tolerated in treatment-naïve and -experienced, genotype 1 or 4 HCV-infected patients with HIV-1 co-infection, including those with cirrhosis. Complete SVR12 data will be presented.

Viral hepatitis: Hepatitis C – d. Clinical (new compounds, resistance)

P0888

EFFECT OF BASELINE FACTORS ON RESPONSE TO THE FIXED-DOSE COMBINATION OF DACLATASVIR (DCV), ASUNAPREVIR (ASV) AND BECLABUVIR (BCV), WITH OR WITHOUT RIBAVIRIN (RBV), IN PATIENTS WITH HCV GENOTYPE 1 INFECTION AND CIRRHOSIS

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Background and Aims: Patient characteristics can influence efficacy and safety outcomes with HCV therapeutic regimens. A phase 3 study (UNITY-2) of the fixed-dose combination of DCV (NS5A inhibitor), ASV (NS3 inhibitor), and BCV (nonnucleoside NS5B inhibitor), with or without RBV, demonstrated high rates of sustained virologic response (SVR) in treatment-naive and experienced patients with HCV genotype 1 infection and compensated cirrhosis. The influence of baseline disease characteristics and demographic variables on outcomes from this study was investigated.
Methods: The 202 enrolled patients were treatment-naive (N = 112) or treatment-experienced (N = 90) adults with HCV genotype 1 infection and compensated cirrhosis. 74% of patients had GT1a infection; 26% had GT1b. All patients received a twice-daily fixed dose combination of DCV 30 mg, ASV 200 mg, and BCV 75 mg, ± weight-based RBV, for 12 weeks. The primary endpoint was SVR at posttreatment week 12 (SVR12).
Results: Overall, 93% of patients achieved SVR12, with higher response rates in patients treated with DCV/ASV/BCV combined with RBV and in patients with GT1b subtype. Other baseline factors such as age, gender, BMI, IL28B genotype, HCV RNA >800,000 IU/mL, and platelet count $<100\times10^9$/L did not appear to impact SVR12 rates in either cohort (Table). Similarly, race did not appear to influence SVR12 rates; of the 20 black patients enrolled in the study, all 20 achieved SVR12. Prior null response to pegIFN did not significantly impact outcome in the treatment-experienced cohort. Baseline resistance-associated variants (RAVs) in NS5A (28, 30, 31, 93) or NS3 (168) did not impact SVR: 26/28 patients with NS5A RAVs and 2/2 patients with NS3 RAVs achieved SVR12. NS5B-P495 was not detected at baseline. Among patients with virologic failure, RAVs were detected in NS5A (Q30, Y93, L31), NS3 (R155, D168) and NS5B (P495, P421).
Conclusions: The all-oral fixed-dose combination of DCV, ASV, and BCV achieved high rates of SVR12 in patients with HCV genotype 1 infection and compensated cirrhosis. The strongest predictors of SVR12 were GT1b and use of RBV. Factors often associated with reduced responses to less potent regimens, such as advanced age, black race, prior null response to pegIFN, high baseline HCV RNA levels, low platelet counts, non-CC IL28B genotype, and baseline NS5A or NS3 RAVs did not appear to impact SVR12 rates.

Subgroup, n/N (%)	Treatment-naive		Treatment-experienced	
	DCV/ASV/BCV N = 57	DCV/ASV/BCV + RBV N = 55	DCV/ASV/BCV N = 45	DCV/ASV/BCV + RBV N = 45
Overall	53/57 (93)	54/55 (98)	39/45 (87)	42/45 (93)
GT 1a	36/40 (90)	38/39 (97)	30/35 (86)	32/35 (91)
GT 1b	17/17 (100)	15/15 (100)	9/10 (90)	10/10 (100)
Age <65 years	39/43 (91)	45/46 (98)	36/42 (86)	31/34 (91)
Age ≥65 years	14/14 (100)	9/9 (100)	3/3 (100)	11/11 (100)
Males	37/39 (95)	34/35 (97)	26/32 (81)	25/27 (93)
Females	16/18 (89)	20/20 (100)	13/13 (100)	17/18 (94)
White race	45/49 (92)	46/46 (100)	35/41 (85)	34/37 (92)
Black race	6/6 (100)	6/6 (100)	2/2 (200)	6/6 (100)
Other race	2/2 (100)	2/3 (67)	2/2 (100)	2/2 (100)
BMI <30 kg/m^2	44/46 (96)	36/36 (100)	27/30 (90)	25/28 (89)
BMI ≥30 kg/m^2	9/11 (82)	18/19 (95)	12/15 (80)	17/17 (100)
HCV RNA <800K IU/mL	10/10 (100)	14/14 (100)	2/2 (100)	4/4 (100)
HCV RNA ≥800K IU/mL	43/47 (92)	40/41 (98)	37/43 (86)	38/41 (93)
IL28B CC (rs12979860)	11/13 (85)	18/18 (100)	13/15 (87)	9/9 (100)
IL28B CT or TT (rs12979860)	41/43 (95)	36/37 (97)	26/30 (87)	33/36 (92)
Platelets $<100\times10^3$/L	8/8 (100)	17/17 (100)	15/18 (83)	10/10 (100)
Platelets $\geq100\times10^3$/L	44/48 (92)	37/38 (97)	24/27 (89)	32/35 (91)
Prior response to IFN regimens				
Null response	NA	NA	18/19 (95)	16/16 (100)
Partial response	NA	NA	4/6 (67)	2/2 (100)
Relapse	NA	NA	7/8 (88)	7/8 (88)
Other [a]	NA	NA	9/11 (82)	13/15 (87)
Other prior HCV treatment [b]	NA	NA	1/1 (100)	4/4 (100)

[a] Breakthrough, IFN intolerant, HCV never undetectable, prior treatment response missing or could not be categorized.
[b] Other treatments included amantadine, alisporivir, mericitabine, GS9450, thymosin, and milk thistle.

P0889

EFFECT OF BASELINE FACTORS ON RESPONSE TO THE FIXED-DOSE COMBINATION OF DACLATASVIR (DCV), ASUNAPREVIR (ASV) AND BECLABUVIR (BCV) IN NON-CIRRHOTIC PATIENTS WITH HCV GENOTYPE 1 INFECTION

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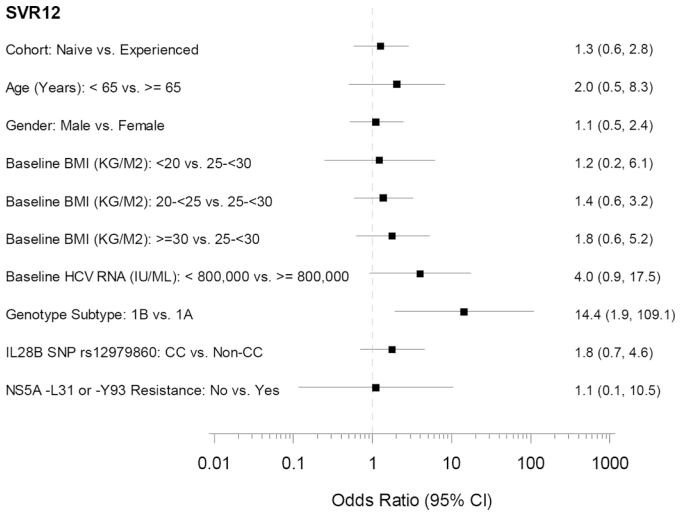
Background and Aims: The all-oral fixed-dose combination of daclatasvir (DCV; pangenotypic NS5A inhibitor), asunaprevir (ASV; NS3 inhibitor), and beclabuvir (nonnucleoside NS5B inhibitor) was evaluated without ribavirin in non-cirrhotic HCV genotype 1-infected patients in the Phase 3 UNITY-1 study. The influence of baseline disease characteristics and demographic variables on outcomes from this study was investigated.

Methods: This study enrolled 415 adult HCV genotype 1-infected patients without cirrhosis, who were either treatment-naive (N = 312) or treatment-experienced (N = 103). All patients received a twice-daily fixed-dose combination of DCV 30 mg, ASV 200 mg, and BCV 75 mg for 12 weeks. For this analysis, baseline characteristics as predictors of SVR at posttreatment week 12 (SVR12) are assessed using a multivariate logistic regression model.
Results: Overall, SVR12 was achieved by 92% of treatment-naive patients and 89% of treatment-experienced patients. Patients with genotype 1b infection were most likely to achieve SVR12 (Figure; Odds Ratio [OR] 14.4; CI 1.9–109.1). There was a trend toward greater likelihood of SVR12 in patients with baseline HCV RNA <800,000 IU/ml (OR 4.0, CI 0.9–17.5), age <65 years (OR 2.0, CI 0.5–8.3), higher body mass index (BMI; OR 1.8, CI 0.6–5.2) and IL28B genotype CC (OR 1.8, CI 0.7–4.6). Prior treatment experience, gender and NS5A variants L31 or Y93 at baseline were not predictive of SVR. Race did not appear to influence SVR12 rates; of the 41 black patients enrolled in the study, 38 (93%) achieved SVR12.
Conclusions: The all-oral, fixed-dose combination of DCV, ASV, and BCV achieved high rates of SVR12 in non-cirrhotic patients with HCV genotype 1 infection. Genotype 1b was the strongest predictor of SVR12. Baseline factors that have previously been shown to impact response to anti-HCV therapies, such as age, race, prior treatment experience, BMI or high baseline HCV RNA had minimal impact on SVR12 rates in this study.



P0890
NOVEL CYCLOPHILIN INHIBITOR CPI-431-32 SHOWS BROAD SPECTRUM ANTIVIRAL ACTIVITY BY BLOCKING REPLICATION OF HCV, HBV AND HIV-1 VIRUSES

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Background and Aims: HCV/HBV-related liver disease is the main cause of morbidity and mortality of HIV-1 patients co-infected with HCV and/or HBV. Despite the recent advent of anti-HCV DAAs, the treatment of HCV/HBV/HIV-1 co-infected patients remains a challenge, as these patients are less responsive to treatment, have higher rates of re-infection, and develop liver fibrosis, cirrhosis and liver cancer more often than mono-infected patients. In this study, we used a novel in vitro co-infection model to demonstrate that CPI-431-32, a novel cyclophilin A (CypA) inhibitor, simultaneously blocks replication of HCV, HBV and HIV-1 in human cells.
Methods: Using a unique and novel *in vitro* co-infection model, we examined whether CPI-431-32 interferes (i) with HCV RNA synthesis of isolated replication complexes using a quantitative replicase assay, (ii) with early steps of viral replication of HIV-1 primary isolates, and (iii) with HBV virus entry (NTCP binding) and viral replication. We also tested by ELISA the CPI-431-32 inhibition of the interaction between CyPA and the viral proteins NS5A and $p24^{gag}$ of HCV and HIV-1 viruses, respectively, to explore the mechanism of action of this drug.
Results: We found that CPI-431-32 blocked viral replication of HCV, HBV and HIV-1. CPI-431-32 was more effective than alisporivir (another CyPA inhibitor) inhibiting replication of each of these three viruses. We also demonstrated that CPI-431-32 blocks nuclear import of HIV-1 virus. CPI-431-32 blocked binding of CYPA to HCV NS5A and HIV-1 $p24^{gag}$ more efficiently than alisporivir, and was more effective than alisporivir against established resistant-variants of HIV-1. No other antiviral agent tested in our assays was able to show such a broad-spectrum of antiviral activity.
Conclusions: The unique broad-spectrum of antiviral activity shown by CPI-431-32 might be related to the role of CyPA in viral infection. CPI-431-32 prevents binding of CyPA, a protein-folding enzyme to viral proteins such as HCV NS5A and HIV $p24^{gag}$, which are critical for HCV and HIV-1 replication. We hypothesize that the inhibition of CyPA by CPI-431-32 is responsible for its broad-spectrum antiviral activity by preventing activation of specific viral proteins required for viral replication. Overall, this study suggests that CPI-431-32, a novel CyPA inhibitor, could potentially become a candidate medicine for the treatment of HIV-1 patients co-infected with HCV and/or HBV.

P0891
RESISTANCE ANALYSIS OF VIROLOGIC FAILURES IN HEPATITIS C GENOTYPE 1 INFECTED PATIENTS TREATED WITH GRAZOPREVIR/ELBASVIR +/− RIBAVIRIN: THE C-WORTHY STUDY

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Background and Aims: Grazoprevir (GZR) is a potent HCV NS3/4A inhibitor and Elbasvir (EBR) is a potent NS5A inhibitor. The Phase 2 C-WORTHY study evaluated the efficacy and safety of GZR/EBR, given once daily, ± ribavirin (RBV), in 471 HCV genotype (GT) 1 infected subjects (36% cirrhotic). In this population, administration of GZR/EBR for 12 or 18 weeks, +/− RBV resulted in SVR12 in 87–100% of subjects. An 8 week regimen resulted in SVR12 in 80% of subjects. We evaluated the frequency of NS3 and NS5A RAVs at baseline and at the time of failure.
Methods: Plasma samples (>1,000 IU/mL) from all patients at baseline and patients who met the criteria for virologic failure (VF) were evaluated by population sequencing of NS3/4A and NS5A, with selected samples further analyzed by clonal sequencing (≥40 clones) or whole genome Next Generation Sequencing (NGS; Illumina) in duplicate.
Results: Excluding 9 early discontinuations due to reasons other than VF, 22/462 (5%) non-SVR_{12} subjects met the protocol defined criteria for VF including 1 subject who was dually infected with GT1b/2b prior to treatment. Post-baseline resistance-associated variants (RAVs) at NS3, Y56H, A156T/G, D168A/E/N/Y, were noted in 13/22 (59%); 1 NS3 RAVs was detected at baseline in 1 subject by NGS. The prevalence of NS5A at VF was high; 20/22 (91%) samples were detected with M28G/T, Q30H/R, L31M/V, H58D and Y93H/N mutations. NGS analysis of baseline samples showed that 13/22 (59%) non-SVR subjects were infected with NS5A RAVs prior to treatment. Clonal sequencing revealed linked mutations among NS3 RAVs and among NS5A RAVs. RAVs containing dual/multiple

mutations had significantly higher resistance comparing to those with single mutations in HCV replicons.
MK-5172 is active against most of these RAVs and the presence of baseline NS3 RAVs did not affect treatment outcomes. The presence of NS5A baseline RAVs by population sequencing was associated with lower SVR (Table).

Gene	Patients with baseline RAVs, n/N (%)	SVR 12, n/N (%)	
		Patients with baseline RAVs	Patients without baseline RAVs
NS3	151/455 (33%)	141/151 (93%)	293/304 (96%)
NS5A	55/450 (12%)	45/55 (82%)	384/395 (97%)

Conclusions: The combination of GZR/EBR has high SVR rates in a diverse HCV infected population including null responders, cirrhotics and HIV/HCV co-infected patients. Virologic failure is associated with NS3 and NS5A RAVs; many of the RAVs identified at failure also pre-existed at baseline as uncovered by NGS.

P0892
IMPACT OF HCV INFECTION IN A HIV COHORT FOLLOWED OVER 18 YEARS: PAST AND PRESENT

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Background and Aims: This study evaluates the impact of HCV infection in a cohort of HIV infected patients followed over 16 years and trends in hospitalizations and mortality.
Methods: A cohort of HIV-infected patients was followed at our institution between 1996–2013. Epidemiological, clinical and main data related with hospitalizations and mortality was recorded. Data has been analyzed considering HIV-mono and HIV/HCV populations and two periods of nine years each.
Results: A total of 2257 HIV infected patients (857 HIV/HCV co-infected) were retrospectively examined over 16 years. Overall, 76% were men, median age of 31 (25–37) years and 57% have an AIDS event during the follow-up. Patients under antiretroviral therapy (ART) was similar in HIV/HCV and HIV-mono in both periods (>87%). Main results are depicted in the table.

Variable	1996–2004			2005–2013		
	HIV	HIV/HCV	p	HIV	HIV/HCV	p
N patients (%)	905 (55)	730 (45)		997 (59)	694 (41)	
Follow-up (years)	6.8±4.7	9.4±4.8	0.001	11.6±4.3	16.4±4.8	0.001
Mortality (%)	311 (34)	275 (37)	0.085	178 (18)	189 (27)	<0.001
Infectious (%)	172 (55)	102 (37)	0.040	60 (34)	41 (22)	0.035
Tumoral (%)	62 (20)	27 (10)	0.020	56 (31)	34 (18)	0.020
Cardiovascular (%)	17 (5)	8 (3)	0.045	23 (13)	18 (9)	0.080
Liver-related mortality (%)	7 (2)	74 (27)	<0.001	1 (1)	72 (38)	<0.001
Hospitalizations	1899	1398	0.110	1297	1458	0.050
Infectious (%)	845 (44)	544 (39)	0.060	454 (35)	517 (35)	0.220
Liver-related (%)	59 (3)	119 (8)	0.001	46 (3)	183 (13)	<0.001

Overall, HIV/HCV co-infected population have significant lower CD4 counts (cell/mm3) in both periods (505 vs 312 and 542 vs 322; $p < 0.001$, respectively). A lower rate of patients achieved HIV-RNA <50 cop/mL in the second period compared with HIV-mono (59.2 vs 56.4 and 75.3 vs 67.2; $p = 0.03$, respectively). In this population, a low rate of patients received HCV treatment although was higher in the second period (6.9% vs 11.5%, $p = 0.01$). Consequently, a higher proportion of patients achieved sustained virological response (SVR) in the second period (61% vs 70%, $p = 0.03$). The rate of liver-related mortality was significantly higher in HIV/HCV compared with HIV-monoinfected with an increase in the second period ($p = 0.02$). Moreover, hospitalizations related with liver-disease were higher in HIV/HCV and were higher in the second period ($p = 0.04$). Among patients achieving SVR, only 3.2% and 3.6% in both periods, respectively, had a liver-related hospitalization and none died.
Conclusions: HCV infection has a negative impact on HIV outcome related with lower CD4 recovery and lower rates of virological success in HIV/HCV patients under ART. However, HCV infection does not impact on the mortality related with infectious, tumoral or cardiovascular diseases and only increase the rate of liver-related mortality in HIV/HCV patients compared with HIV-monoinfected. Moreover, HCV infection significantly increased liver-related hospitalizations and mortality on the long-term. Considering the low rate of patients receiving HCV treatment in this cohort, these data confirm the harmful contribution of uncontrolled HCV infection in HIV/HCV infected population.

P0893
RESISTANCE ANALYSIS OF TREATMENT-EXPERIENCED GENOTYPE 1 AND 3 HCV INFECTED PATIENTS TREATED WITH SOFOSBUVIR IN COMBINATION WITH GS-5816 +/− RIBAVIRIN FOR 12 WEEKS

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Background and Aims: The effect of pretreatment NS5A variants on the response to NS5A inhibitors appears to be dependent on the specific variants present, the HCV genotype and the resistance profile of the NS5A inhibitor. In this study we evaluated the impact of preexisting substitutions at resistance associated variant (RAV) positions on treatment outcome and emergence of RAVs at relapse in treatment experienced GT1 or 3 infected patients receiving sofosbuvir (SOF) 400 mg with the investigational NS5A inhibitor GS-5816 (25 mg or 100 mg) ± ribavirin (RBV) for 12 weeks in study GS-US-342-0109.
Methods: NS5A and NS5B deep sequencing analysis was performed for all patients (n = 321; GT 1=111; GT3=210) at pretreatment and for patients who did not achieve SVR12 at failure timepoints. Variants at known NS5A RAV positions as well as the NS5B SOF RAV S282T and treatment-emergent variant (TEV) positions L159 and V321 were analyzed.
Results: Nineteen GT1 infected patients (16%; 12 GT1a, 7 GT1b) had pretreatment NS5A RAVs (Q30H/R, L31M/V, H58D and Y93C/F/H) with 2/19 patients experiencing relapse compared to no relapse in patients without pretreatment RAVs. GT3 NS5A naturally encodes K24S, Q30A, H58P and A92E when compared to GT1a. Forty-five GT3 infected patients (21%; 43 GT3a, 2 GT3b) also had pretreatment NS5A variants at RAV positions (M28T, A30K/M/R/S/T/V, L31M and Y93H). Of these 45 patients, 6/27 (22%) and 1/18 (6%) relapsed following treatment with SOF+GS-5816 25 mg or 100 mg±RBV respectively compared to 14/78 (18%) and 2/87 (2%) without RAVs. All 3 patients (2 GT1; 1 GT3a) with pretreatment NS5B L159F achieved SVR. Twenty-five patients experienced virologic relapse (2 GT1a, 22 GT3a, 1 GT3b) with 20 treated with SOF+GS-5816 25 mg ± RBV, and 5 treated with SOF+GS-5816 100 mg ± RBV. All relapse patients were observed to have NS5A RAVs at posttreatment with Y93H the predominant RAV observed in GT3 subjects, which displays >100 reduction in susceptibility to GS-5816. GT1a patients had Q30H or L31V enrich from low levels at pretreatment to the major quasispecies at relapse. Neither S282T nor other SOF-TEVs in NS5B were detected in any of the patients at relapse.
Conclusions: SOF+100 mg GS-5816 administered for 12 weeks results in high SVR within treatment-experienced GT1 and 3 infected patients irrespective of pretreatment NS5A RAVs. NS5A resistance but not SOF-resistance was detected in relapse patients.

P0894

PREVALENCE OF PRE-TREATMENT NS5A AND NS5B RESISTANCE ASSOCIATED VARIANTS AND GENETIC VARIATION WITHIN HCV SUBTYPES ACROSS DIFFERENT COUNTRIES

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Background and Aims: Prevalence of resistance associated variants differs by HCV subtype and geographical region. In this study, a comprehensive pooled analysis of HCV sequences was used to investigate genetic variation within HCV subtypes from multiple countries across genotypes 1–6. The prevalence of pre-treatment NS5A and NS5B resistance associated variants was also evaluated across different countries.
Methods: NS5A and NS5B sequencing analyses were performed from >3000 and >7800 patients across 14 and 22 countries, respectively. The presence of NS5A resistance associated variants (K24G/N/R, M28A/G/T, Q30any, L31any, H58D, A92K/T, Y93any in genotype 1a and L31any, A92K, Y93any in genotype 1b) and NS5B NI-associated variants (L159F, S282T, L320F, V321A in genotypes 1–4) were evaluated. Maximum likelihood phylogenetic analyses were performed for a subset of full length NS5A and NS5B sequences from multiple countries.
Results: Prior to treatment, NS5A resistance associated polymorphisms ranged between 6–17% in GT1a and 8–17% in GT1b HCV infected patients from investigated countries (Table). No NS5B S282T or V321A variants were found at baseline. Baseline L320F was only found in 2% of GT1b NZL patients. L159F was mostly found in GT1b, with the highest prevalence in Russia, 34%, followed by Spain, 32%, and the lowest in Japan and Taiwan, 1% and 0%, respectively.
Phylogenetic analyses within subtypes 1a, 1b, 2a, 3a, 4a and 6a revealed a distinct clustering within GT1b suggesting that GT1b is genetically different in several countries. Geographical clustering in other subtypes was less pronounced.

Country	% of GT1a patients with BL NS5A RAVs	% of GT1b patients with BL NS5A RAVs	% of GT1b patients with BL NS5B L159F
Australia	8% (N = 75)	16% (N = 31)	29% (N = 14)
Germany	7% (N = 74)	8% (N = 87)	25% (N = 75)
Spain	6% (N = 33)	14% (N = 22)	32% (N = 34)
France	8% (N = 62)	11% (N = 35)	16% (N = 19)
Italy	17% (N = 41)	13% (N = 67)	20% (N = 51)
Russia	ND	ND	34% (N = 64)
Japan	ND	16% (N = 329)	1% (N = 330)
Korea	ND	16% (N = 85)	5% (N = 86)
Taiwan	ND	12% (N = 68)	0% (N = 67)
USA	8% (N = 2520)	12% (N = 730)	4% (N = 982)
New Zealand	14% (N = 152)	17% (N = 36)	4% (N = 45)

N, number of sequences investigated; ND, no data.

Conclusions: The prevalence of pre-treatment NS5A resistance associated variants varied moderately between different countries and ranged between 6–17%. The major NS5B RAV S282T was not observed in any pre-treatment sample, however L159F showed geographical clustering within GT1b HCV with highest prevalence in Russia and Spain and lowest in Japan and Taiwan.

P0895

COMPARISON OF THREE SEQUENCING METHODS COMMONLY USED IN HEPATITIS C VIRUS RESISTANCE ANALYSIS: POPULATION-BASED VS. CLONAL VS. ULTRA DEEP SEQUENCING

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Background and Aims: Today, treatment of chronic hepatitis C virus (HCV) infection consists of at least one direct acting antiviral drug, targeting the HCV NS3, NS5A, or NS5B gene. Despite high SVR rates, in some patients selection of resistant variants causes treatment failure. Thus, generation of multi resistant viral variants may become a practical problem. We compared three commonly used sequencing methods (population-based, clonal, and next generation sequencing; NGS) with markedly different sensitivities for variant calling, to find out which method provides the best and most effective resolution of HCV quasispecies.
Methods: From 20 treatment-naïve chronic HCV genotype 1 infected patients, a 6kb-sized PCR product covering NS3-NS5B was analyzed. A population-based and a clonal Sanger sequencing approach were performed, as well as ultra-deep sequencing (454 GS FLX+ system). For clonal investigation a mean of 33 (range 19–45) clones was analyzed. High quality reads were mapped to the HCV-J/HCV-H77 reference genomes. Nucleotide variant distributions were called for each variant position using the SAMtools suite. Sequence data was clipped using the phred-quality scores. Remaining reads were mapped to the subgenotype reference genome using SMALT. The 454 coverage varied between approx. 500 and approx. 3750 reads per position.
Results: The sensitivity of population-based sequencing was clearly shown to be limited. Only 36% of variants with frequencies up to 10% were called. At a 10% cut-off, sensitivity of direct sequencing vs. NGS is 38%, 37%, and 34% for NS3, NS5A, and NS5B, respectively. With the clonal approach sensitivity is 76%, 87%, and 84% for NS3, NS5A, and NS5B, in comparison to NGS, respectively. Specificity is at a high level for both methods and all genes (97% for clonal and 99.9% for direct sequencing).
Conclusions: Clonal sequencing lies qualitatively between population-based and deep sequencing, but it is much more expensive and time-consuming. Population-based sequencing is cheap and fast, but bears the danger of missing important variants associated with DAA resistance. NGS shows the highest sensitivity and becomes more and more affordable, but currently is also a very laborious approach, which might be difficult to integrate in clinical routine.

P0896

LONG-TERM FOLLOW-UP ANALYSIS OF RAVS IN HCV NS3, NS5A, AND NS5B IN DAA THERAPY FAILURE PATIENTS

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Background and Aims: Despite high SVR rates to different direct acting antiviral (DAA) drug based regimens for hepatitis C virus (HCV) infection, viral breakthrough or relapse is observed and selection of resistant variants is a major cause for treatment failure. Thus, generation of multi resistant viral variants may become a practical problem. Data on long-term evolution of resistance associated variants (RAVs) especially for NS5A and NS5B inhibitors in patients (pts) with treatment failure are sparse. In the present study, we show first data about long-term persistence and evolution of resistance variants in HCV NS3, NS5A, and NS5B from pts with failure to DAA-based therapies.

Methods: Population-based sequencing was performed from the HCV NS3, NS5A, and NS5B region. For NS3, 12 pts with 2–14 time points up to 2 years after telaprevir triple therapy start were included. For analysis of the NS5A/5B genes, 6/5 pts with 3–6/2–5 time points up to 3 years after daclatasvir/ledispasvir/ombitasvir therapy and until follow-up (FU) week 12 of deleobuvir treatment, respectively, were available.
Results: In 9/12 pts NS3 PI RAVs could be detected by population-based sequencing after stopping treatment. After 1-year FU, in 5/10 pts single RAVs (V36A/L, T54S/A, R155K) were still detectable, while at 2 years FU, in 2/4 pts T54A or R155K could be observed.
Five patients who were treated with an NS5A inhibitor showed variants associated with treatment failure. At 1-year FU in 4/6 pts one or more RAVs were observed (Q30R, L31M, H54A, H58P in genotype 1a and L31M/I, H58S, Y93H in genotype 1b). The Q30R/L31M double variant was still detectable in 1/2 pts after 2 years FU.
No RAVs could be detected in 5 patients who received the non-nucleoside NS5B inhibitor deleobuvir at treatment failure and follow-up so far.
Patterns of evolution, persistence and re-occurrence without any selection pressure present, differed in all groups between the single patients.
Comparison with deep sequencing will be presented at the meeting.
Conclusions: Variable rates of persistence of RAVs in patients with failure to NS3, NS5A and NS5B inhibitors have been observed. While generally RAVs to protease inhibitors tend to decline and RAVs to NS5A inhibitors persist in single patients, highly resistant variants have been observed up to 3 years after treatment failure.

P0897

EFFICACY OF SIMEPREVIR AND SOFOSBUVIR FOR TREATMENT OF CIRRHOTIC PATIENTS WITH GENOTYPE 1 CHRONIC HEPATITIS C – A SINGLE CENTER EXPERIENCE

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Background and Aims: In the COSMOS study, treatment of genotype (G)1 hepatitis C (HCV)-related cirrhosis (N = 18) for 12 weeks using the HCV protease inhibitor (PI), Simeprevir (SIM), and the polymerase inhibitor, Sofosbuvir (SOF), either with (N = 11) or without (N = 7) ribavirin (RBV), reported a high cure rate (SVR12 in 10/11 [91%] and 6/7 [86%]) and favorable safety profile. Because interpretation is limited by sample size, we evaluated the clinical efficacy and safety of this regimen in 78 cirrhotic patients in real-world use.
Methods: Retrospective analysis of cirrhotic G1 HCV patients treated daily with SIM 150 mg and SOF 400 mg. Post liver transplant cases were excluded. The presence of cirrhosis was based on firm clinical, radiographic or pathological evidence, or FibroSURE test results. Laboratory tests were obtained every 2–4 weeks. HCV RNA was assayed by real-time PCR (COBAS® LLD:15 IU/mL).
Results: Patient demographics and clinical characteristics are shown in table 1. Seventy-eight cirrhotic patients were treated: 73 were treated for 12 weeks, 2 for 4.5 weeks, 1 for 6.5 weeks, 1 for 16 weeks and 1 for 24 weeks. Only 2 received RBV. After starting treatment, viral load (VL) was undetectable at week 2 in 20 of 72 tested patients (28%); data was unavailable for 6 patients. Sixty-three (81%) patients had undetectable VL at week 4. Of 76 patients tested all had undetectable VL at end of treatment (EOT); for 2 patients EOT VL was unavailable. Fifty-two (67%) patients achieved SVR12 while 21 (27%) relapsed. For 5 patients week 12 VL was not obtained due to lost follow-up (N = 2) or a shorter duration of treatment (3). Only 1 patient had on-treatment virologic breakthrough despite reported compliance. Three patients discontinued treatment prematurely due to severe hyperbilirubinemia (N = 2) or hepatic decompensation (N = 1). At start of treatment, mean MELD score was 9.5, bilirubin 1.3, ALT 90, platelets 105. At EOT, mean MELD was 10, bilirubin 1.6, ALT 29, platelets 106.

Table: Demographic and clinical characteristics at baseline and after SIM-SOF: N = 78

	Cohort (N = 78) [a]	Relapsers (N = 21)	SVR12 (N = 52)
Male gender, N (%)	49 (62.8%)	15	N/A
Ethnic origin (N, %)		N/A	N/A
Caucasian	54 (69.2%)		
African American	4 (5.1%)		
Asian	4 (5.1%)		
Hispanic & Latino	2 (2.6%)		
Unknown race	14 (17.9%)		
Age, mean, median (SD)	62.8, 62 (6.6)	59.9, 60 (4.4)	N/A
BMI, mean, median, (SD)	28.5, 26.5 (6.2)	29.7, 27.2 (7.3)	N/A
MELD score, mean	9	11, 2, 3	N/A
Baseline HCV RNA (IU/ml)	N/A	1,485,759	N/A
HCV GT, N (%)			N/A
1a	47 (60.3%)	15	
1b	31 (39.7%)	6	
Treatment history, N (%)			N/A
Treatment naïve	31 (39.7%)	7 (33.3%)	
Prior interferon therapy	47 (60.3%)	14 (42.4%)	
Prior PI therapy	5 (6.4%)	2 (9.5%)	
No response	28 (53.8%)	8 (38.1%)	

N/A, Not yet available: data pending.
[a] 5 patients lost to follow-up.

Conclusions: Twelve weeks of SIM-SOF therapy for G1 HCV cirrhosis cured no more than 67% of patients, indicating a significantly higher relapse rate than previously reported. Overall tolerability and relative safety was acceptable.

P0898

TD-6450, A NEXT GENERATION ONCE-DAILY NS5A INHIBITOR, HAS POTENT ANTIVIRAL ACTIVITY FOLLOWING A 3-DAY MONOTHERAPY STUDY IN GENOTYPE 1 HCV INFECTION

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Background and Aims: TD-6450 is a next generation HCV NS5A inhibitor with superior in vitro potency against resistance-associated variants (RAVs) encountered with first-generation NS5A inhibitors. This study evaluated the safety, pharmacokinetics (PK) and antiviral activity of TD-6450 following multiple oral doses in HCV patients.
Methods: This was a double-blind, randomized, placebo-controlled study in treatment-naïve, non-cirrhotic HCV patients. In the dose ranging portion of the study, patients with GT-1a infection were randomized to receive multiple ascending oral doses of TD-6450 [60 mg to 240 mg daily (QD)] or placebo for 3 days. Additional cohorts of GT-1b, GT-2 and GT-3 HCV patients were administered TD-6450 240 mg QD and one GT-1a cohort was administered 240 mg twice daily (BID) for 3 days. The antiviral activity of TD-6450 was assessed using the COBAS®Ampliprep/COBAS®Taqman® HCV Test, 2.0 (limit of detection, LOD = 15 IU/mL) for up to 28 days after the first dose of TD-6450.
Results: Twenty-four patients with GT-1a infection were evaluated following daily oral doses (60 mg to 240 mg) of TD-6450 (n = 21)

or placebo (n = 3) for 3 days. The mean baseline viral loads ranged from 6.1 to 6.3 log10 IU/mL. All GT-1a patients in these cohorts had a maximum HCV RNA viral load decline of at least 3 log10 IU/mL. The HCV RNA results are presented in Table 1. Seven patients (3 in the 120 mg and 4 in the 240 mg groups) had at least one HCV RNA viral load which fell below the LOD and two of these patients had persistent unmeasurable HCV-RNA at Day 28. All doses of TD-6450 were generally well tolerated after three doses and for the 28-day observation period. There were no serious adverse events and no patient discontinuations. There was no pattern of clinical adverse events or laboratory abnormalities related to treatment. The PK of TD-6450 was similar to that observed in healthy subjects and consistent with QD dosing. The evaluation of TD-6450 in GT-1a (BID), GT-1b, GT-2, and GT-3 HCV patients is currently ongoing but dosing has completed and results will be presented.

Table 1. TD-6450 median maximum decline in log_{10} HCV RNA in GT-1a HCV patients (n = 7)

TD-6450 dose	Median maximum decline in HCV RNA (min, max)
60 mg QD	3.9 (3.49, 4.36)
120 mg QD	4.6 (3.11, 5.91)
240 mg QD	4.9 (3.40, 5.80)

Conclusions: TD-6450 was generally well tolerated at all doses evaluated and demonstrated potent dose-dependent antiviral activity in treatment-naïve HCV GT-1 infected patients.

P0899

PRECLINICAL PROFILE OF THE PAN-GENOTYPIC HCV NS3/4A PROTEASE INHIBITOR GS-9857

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Background and Aims: GS-9857 is a pan-genotypic HCV NS3/4A protease inhibitor (PI) in development for the treatment of chronic HCV infection. During a 3-day monotherapy study in GT1–4 patients, GS-9857 was well-tolerated and resulted in median HCV RNA reductions of >3 log_{10} IU/mL at the 100 mg dose. Here we profile preclinical properties of GS-9857 including in vitro potency, selectivity, activity against resistance associated variants (RAVs) as well as pharmacokinetic properties in preclinical species.
Methods: The binding affinity of GS-9857 was tested against wild-type GT1b and GT3a NS3 proteases in biochemical assays. Antiviral activity was studied in 3-day HCV replicon assays using stable or transient replicons encoding the NS3 protease gene from GTs 1–6. Plasma and liver pharmacokinetics were assessed in Sprague-Dawley rats and beagle dogs after oral and intravenous administration.
Results: GS-9857 has picomolar inhibitory activity against GT1b and GT3a NS3 proteases (Ki values of 38 pM and 63 pM, respectively), potent antiviral activity against representative GT1–6 HCV replicon strains (table), and low cytotoxicity (CC50 18–21μM). GS-9857 has less than a 2.5 fold shift in antiviral activity in vitro against the clinically relevant HCV PI RAVs 1a Q80K, 1a R155K, 1b D168A, 1b D168E, and 1b D168V. Good oral bioavailability was observed with GS-9857 in rats and dogs (27–83%) with plasma half-lives of 1.6 hours and 4.1 hours, respectively. The liver concentrations of GS-9857 are approximately 500-fold (rat) and 55-fold (dog) higher than the corresponding plasma concentrations, demonstrating that GS-9857 preferentially distributes to the liver. GS-9857 is also stable in human microsomes and hepatocytes.
Conclusions: GS-9857 is a potent pan-genotypic HCV NS3/4A PI with antiviral activity against HCV GTs 1–6 and an improved resistance profile compared to previous HCV PIs. Importantly, GS-9857 has similar antiviral activity against replicons of all GTs including GT3 (<2-fold difference in EC50 between GT1 and GT3). GS-9857 has a favorable pharmacokinetic profile in preclinical species and is stable in human hepatocytes. The preclinical profile of GS-9857 supports its development as part of a pan-genotypic regimen to treat chronic HCV infection.

P0900

CONSIDERATION OF VIRAL RESISTANCE FOR OPTIMIZATION OF DIRECT ANTIVIRAL THERAPY OF CHRONIC HEPATITIS C

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Background and Aims: For chronic hepatitis C virus (HCV) genotype (GT) 1 infected patients, combination therapies of a nucleoside NS5B inhibitor [Sofosbuvir (SOF)] with a NS3 protease inhibitor (Simeprevir, SMV) or a NS5A inhibitor (Daclatasvir, DCV and Ledipasvir, LDV) are available. Alternative options are DCV plus the NS3 inhibitor Asunaprevir (ASV) for GT1b or a triple DAA therapy (NS3, NS5A and non-nucleoside NS5B inhibitor) for GT1 (Paritaprevir, PTV, Ombitasvir, OBV and Dasabuvir, DSV). In the underlying studies, sustained virologic response rates (SVR) were high (up to 99%), but pre-existent resistance-associated variants (RAVs) let to a reduction of SVR rates of a range between 5 and 50%.
Methods: Serum samples of consecutive 312 European patients with a chronic GT 1 HCV infection who were treatment- and DAA-naïve were used for the parallel amplification and population-based sequencing of NS3, NS5A and NS5B genes. NS3 RAVs with relevance for SMV, PTV and ASV were investigated at positions F43, Y56, Q80, R155, A156 and D168. Analysis of NS5A RAVs with relevance for DCV, LDV and OBV occurred at positions M28, Q/R30, L31, P32L, Q54H, H/P58 and Y93. For the non-nucleoside NS5B inhibitor DSV relevant positions C316, S368, Y448, S556 and D559 within NS5B were also examined. Sequencing of SOF RAVs (L159, S282, V321) was not performed as these variants were not detected at baseline and / or were not associated with reduced SOF susceptibility.
Results: Major resistant variants with important differences for HCV subtypes were detected within NS3 in 20.5% of patients and within NS5A and NS5B in 11.5% and 21.5% individuals respectively. For NS3, Q80K was observed in 34.5% and 2.1% of subtype 1a and 1b patients, respectively while other RAVs to second generation protease inhibitors were detected rarely (0.7%). Within NS5A RAVs were observed in 7.0% of subtype 1a and 17.0% of subtype 1b infected patients and Y93H was most prevalent (14.2%, GT1b). NS5B RAVs were found in 2.3% and 44.7% of subtype 1a and 1b patients, while C316N, S556G and combinations of both were most frequently detected (22.7%, 7.1%, 12.8%; GT1b). Considering all three DAA targets all subtype 1a and 98.6% of subtype 1b were wildtype for at least one interferon free DAA regimen currently available.
Conclusions: Baseline resistance testing allows the selection of at least one RAVs-free treatment option for nearly all patients enabling a potentially cost-optimized treatment of chronic hepatitis C.

Table (abstract P0901): Maximal reductions in HCV RNA (log_{10} IU/mL)

	GT1a			GT1b	GT2	GT 3			GT 4
	50 mg	100 mg	300 mg	100 mg	100 mg	50 mg	100 mg	300 mg	100 mg
N	8	8	8	6	6	5	6	5	4
Median	−4.13	−4.38	−3.99	−3.90	−3.60	−1.95	−3.24	−3.56	−4.11
Q1, Q3	−3.87, −4.42	−4.26, −4.55	−3.66, −4.30	−3.76, −4.09	−3.07, −3.66	−1.54, −2.27	−2.75, −3.77	−3.53, −4.13	−3.41, 4.55
Min, Max	−3.77, −4.63	−3.43, −5.05	−2.72, −5.49	−3.51, −4.10	−2.92, −4.17	−1.39, −2.34	−1.59, −5.16	−3.00, −4.25	−3.03, −4.66

P0901

THE PAN-GENOTYPIC NS3/4A PROTEASE INHIBITOR GS-9857 DEMONSTRATES POTENT ANTIVIRAL ACTIVITY IN PATIENTS INFECTED WITH HCV GENOTYPE 1, 2, 3 OR 4 IN A 3-DAY MONOTHERAPY STUDY

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Background and Aims: GS-9857 is a pan-genotypic HCV NS3/4A protease inhibitor (PI) in development for the treatment of chronic HCV infection with potent antiviral activity against HCV genotypes (GT) 1–6 and an improved resistance profile compared to previous HCV PIs. Herein, we describe the safety, pharmacokinetic (PK) and antiviral activity from a 3-day monotherapy study in patients infected with HCV GT 1a, 1b, 2, 3 and 4.

Methods: HCV NS3/4A PI-naive patients without cirrhosis were randomized to receive placebo or GS-9857 once daily for 3 days at doses ranging from 50 mg to 300 mg. Safety was assessed by physical examination, ECG and laboratory tests during treatment and for 1 week post-dosing. GS-9857 PK was assessed on Day 1 and 3. Antiviral activity was evaluated by measuring HCV RNA using the COBAS Taqman HCV Test, v2.0 for use with Ampliprep (LLOQ = 15 IU/mL).

Results: Sixty-nine (69) patients with GT 1a (n = 28, 41%), 1b (n = 6, 9%), 2 (n = 8, 12%), 3 (n = 23, 33%), and 4 (n = 4, 6%) were enrolled in the study. The population was 71% male with a mean age of 50 (range 30–64) and BMI 27.7 kg/m^2 (range 19.4–52.5) and mean HCV RNA of 6.2 log_{10} IU/mL (range 5.8–6.7). The median reductions (log_{10} IU/mL) in HCV RNA are presented in the table. GS-9857 exhibited comparable half-life, linearity and accumulation as observed in healthy volunteers with approximately 4-fold higher plasma exposures at the same dose. No patients discontinued treatment due to an adverse event (AE). All AEs were mild or moderate in severity. The only AE occurring in >5% of patients was diarrhea (n = 2, 6%). There were no clinically significant changes in ECG or vital signs. One patient had a Grade 4 creatine kinase on Day 3 which increased from screening to baseline prior to dosing and resolved without intervention.

Conclusions: During a 3-day monotherapy study in patients infected with HCV GT 1a, 1b, 2, 3 or 4, GS-9857 was well-tolerated and resulted in median HCV RNA reductions of >3 log_{10} IU/mL at the 100 mg dose.

P0902

EXPOSURE-RESPONSE ANALYSES FOR EFFICACY (SVR12) FOR THE DIRECT ACTING ANTIVIRAL REGIMEN OF ABT-450/R, OMBITASVIR WITH DASABUVIR +/− RIBAVIRIN IN SUBJECTS WITH HCV GENOTYPE 1 INFECTION

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Background and Aims: The 3 direct acting antiviral (DAA) regimen (3D) of coformulated ABT-450/r [protease inhibitor identified by AbbVie and Enanta dosed with ritonavir (RTV)] with ombitasvir (NS5A inhibitor) plus dasabuvir (polymerase inhibitor) ± ribavirin (RBV) has completed Phase 3 clinical trials in HCV genotype (GT) 1 infected subjects. The objective of this analysis was to examine the relationships between the steady-state exposure (AUC and C_{trough}) of DAAs and RBV with SVR_{12} following administration of the 3D regimen in five Phase 3 and one Phase 2 clinical trials.

Methods: Graphical and quantitative multivariate logistic regression (LR) analyses were performed using steady state AUC and C_{trough} of DAAs and RBV based on the population-pharmacokinetic models. Graphical analysis was performed using quartiles plots. Since >99% of GT1b-infected subjects [N = 996] achieved SVR_{12}, only data for GT1a-infected non-cirrhotic subjects (N = 615) who received the 3D + RBV for 12 weeks were included in the multivariate LR analyses performed using SAS (version 9.3). Subject-specific covariates, such as age, body mass index, sex, ethnicity (Hispanic/Latino vs. others) and race (Black vs. others), baseline HCV viral load (VL), IL28B genotype, and prior pegIFN/RBV treatment, were included in the analyses.

Results: No trend was observed between the tested DAA or RBV AUCs and % SVR_{12} among GT1b-infected subjects with graphical analyses. Among GT1a-infected subjects, there was no apparent relationship between the tested dasabuvir AUC and % SVR_{12}, but shallow trends were observed for the tested ABT-450, ombitasvir and RBV AUCs. Multivariate LR analyses did not reveal a significant association between the tested ABT-450, dasabuvir or ribavirin AUCs and SVR_{12}, but an increase in the tested ombitasvir AUCs was associated with a slight increase in probability of SVR_{12} ($P < 0.05$); a maximum of ± 25% change in ombitasvir AUC observed in drug interaction studies predicted about ± 1% change in SVR_{12}, which is not clinically significant. A Prior history of non-response to pegIFN/RBV treatment, IL28B genotype, baseline VL, sex, race and Hispanic/Latino ethnicity were not predictive of a change in the probability of SVR_{12}. Similar relationships were observed between the tested DAA or RBV C_{trough} and SVR_{12}.

Conclusions: The results of these analyses indicate that the doses selected for the 3D regimen achieved high SVR_{12} rates across the range of exposures observed in the Phase 3 studies.

P0903

SLOW HCV KINETICS FOLLOWING SOFOSBUVIR + RIBAVIRIN ADMINISTRATION IN REAL-LIFE SETTING OF LIVER TRANSPLANT RECIPIENTS WITH SEVERE RECURRENT HEPATITIS C

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Background and Aims: Hepatitis C Virus (HCV) infection is a leading cause of liver transplantation (LT) and is associated with the worst clinical outcome after LT due to fast and frequent severe HCV recurrence. Our aim was to investigate whether early HCV-RNA kinetics during sofosbuvir (SOF) + ribavirin (RBV) treatment are influenced by HCV-genotype and severity of recurrent HCV hepatitis.
Methods: 34 post-LT patients with severe HCV-recurrence (fibrosing cholestatic hepatitis = 7, cirrhosis = 14, advanced fibrosis F3 by Metavir = 13), infected by HCV genotype 1a, 1b, 2, 3a and 4 (7, 11, 5, 6, 5), received SOF 400 mg/day + RBV 400–1000 mg/day (N = 27) or SOF alone (N = 7), for a median (IQR) of 8 (4–16) weeks. HCV-RNA was quantified at baseline (BL) and weekly during treatment. HCV-RNA kinetics was modeled according to Guedj 2014. Presence of NS5B resistance associated variants (RAVs) was evaluated by Sanger-sequencing. Safety and clinical parameters were also analyzed.
Results: Overall, a slow HCV RNA decay was observed: only 6/34 (18%) patients had undetectable (TND) HCV-RNA values at week 4 (rapid virological response, RVR). No patient infected with genotype 1a, 2 and 4 had RVR. Nine out of 21 evaluable patients (43%) had TND at week 8, and 8/10 (80%) at week 12. Non-cirrhotic patients had a significantly higher BL HCV-RNA compared to patients with cirrhosis (median [IQR] 6.5 [6.2–6.8] Log IU/ml vs 5.7 [5.4–5.9] Log IU/ml, p = 0.001), without different kinetics of HCV-RNA decline and RVR frequencies (18% *vs* 27% respectively, p = 0.6). Failure to achieve RVR was not related to BL viremia (p = 0.2), nor RBV-administration (p = 0.4), but it was associated with slower early HCV-RNA kinetics. Indeed, 17/32 patients had HCV-RNA >100 IU/ml at week 2, and none of them reached RVR vs 33% of patients with HCV-RNA<100 IU/ml (p = 0.01). No baseline-biochemical or clinical parameter significantly correlated with early slow viral kinetics, with the exception of cholesterol levels, that were higher in patients with HCV-RNA >100 IU/ml at week 2 (median [IQR] 156 [143–186] vs 115 [88–160] mg/dl, p = 0.024). No baseline RAVS were detected in all 10 patients analyzed, with the exception of L159F in 1 HCV-1b-naive cirrhotic patient that became RVR.
Conclusions: In the context of an heterogeneous population of patients with severe HCV-recurrence post-LT in real-life setting, at this preliminary phase, the combination of SOF +/− RBV shows an overall slow kinetics of HCV-RNA decay, regardless of BL viremia and, notably, regardless of the type and stage of liver disease.

P0904

RG-101, A NOVEL GalNAc-CONJUGATED INHIBITOR OF MICRORNA-122, DEMONSTRATES SIGNIFICANT VIRAL LOAD REDUCTION AND REDUCES LIVER STEATOSIS IN HUMAN HEPATOCYTE CHIMERIC MICE INFECTED WITH GENOTYPE 1A OR HARD-TO-TREAT GENOTYPE 3A HEPATITIS C VIRUS (HCV)

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Background and Aims: RG-101 is a novel phosphorothiate oligonucleotide that binds and efficiently inhibits microRNA (miR)-122, which is abundantly and specifically expressed in hepatocytes and plays a key role in the replication of the hepatitis C virus (HCV). Preferential targeting to hepatocytes was achieved through conjugation of the oligo to GalNAc through a phosphodiester linker, which results in a pro-drug that is metabolized in the liver to an active unconjugated oligo. A number of studies have been performed demonstrating significantly enhanced pharmacodynamic activity of RG-101 compared to its unconjugated parent oligo in both normal mice and non-human primates at significantly lower concentrations in the liver (Bhat et al., Hepatology 58; 2013).
Methods: Effects of RG-101 on HCV infection were examined using human hepatocyte chimeric mice (PXB-mice) infected with genotype (GT) 1a and hard-to-treat genotype 3a HCV isolates.
Results: Expression levels of ASGPR, receptors on human hepatocytes that bind GalNAc and facilitate cellular uptake of RG-101, were 2–3 fold lower in stably engrafted human hepatocytes compared to fresh hepatocytes prior to transplantation. Pharmacodynamic activity of RG-101, measured by de-repression of endogenous miR-122 target genes, was 3–5 fold lower in PXB-mice compared to wild-type mice after a single subcutaneous (SC) injection, correlating with a 3 fold reduced level of oligo uptake in the liver of PXB-mice at all doses tested. A single SC administration of RG-101 in HCV GT1a-infected PXB-mice produced statistically significant 1 and 1.5 $\log_{10}$ viral titer reductions at 10 and 30 mg/kg with maximum reduction between 10 and 14 days post-dose. Consensus sequencing of virus in the serum at day 42 post-dose showed no mutations in the miR-122 binding sites. In GT3a-infected PXB-mice, a single SC administration of RG-101 produced about 1.5–2 $\log_{10}$ viral load reduction with maximum reduction at 14–17 days. A further 0.5 $\log_{10}$ reduction was observed following a second SC dose given at day 28. Liver steatosis observed in PXB-Mouse livers was dramatically reduced following RG-101 treatment in line with the known role of miR-122 in hepatic lipid metabolism.
Conclusions: Taken together, these studies in PXB-mice provide in vivo proof that RG-101 is effective against multiple HCV genotypes and may have additional benefits beyond viral titer reduction.

P0905

NO SIGNIFICANT INTERACTION AMONG OMBITASVIR/ PARITAPREVIR/RITONAVIR +/− DASABUVIR AND SOFOSBUVIR

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Background and Aims: Paritaprevir [NS3/4A inhibitor identified as a lead compound by AbbVie and Enanta, dosed with ritonavir (r)], ombitasvir (NS5A inhibitor) and dasabuvir (NS5B non-nucleoside polymerase inhibitor) are being developed as an interferon-free direct-acting antiviral agent (DAA)-combination therapy for HCV infection. The 3-DAA (3D) regimen of coformulated ombitasvir/paritaprevir/r + dasabuvir +/− ribavirin (RBV) has completed Phase 3 clinical trials in HCV genotype (GT) 1-infected subjects. The 2-DAA (2D) regimen of ombitasvir/paritaprevir/r +/− RBV has been evaluated in HCV GT1b- and GT4-infected subjects.

The 3D and 2D regimens have the potential for co-administration with sofosbuvir (SOF, nucleoside NS5B inhibitor) in the treatment of HCV. A drug–drug interaction study was conducted to assess the potential for interactions.

Methods: This Phase 1, open-label, multiple-dose, sequential, non-fasting study enrolled 32 healthy subjects into 2 arms; each arm comprised 2 cohorts (Figure 1). Doses of study drugs were: ombitasvir/paritaprevir/r 25/150/100 mg QD, dasabuvir 250 mg BID, and SOF 400 mg QD. Intensive pharmacokinetic assessment was performed on Study Days 7, 14, 15 and 21. The effect of the DAAs on SOF pharmacokinetics and vice versa for each arm was assessed by a repeated-measures analysis using SAS. Safety was evaluated through assessment of adverse events (AEs), vital signs, ECGs and clinical laboratory tests.

Results: Ombitasvir, paritaprevir and dasabuvir C_{max}, AUC and C_{24} during co-administration with sofosbuvir as either the 3D or 2D regimen were similar (≤20% change) to 3D or 2D alone. Sofosbuvir C_{max} and AUC were 61% and 112% higher, respectively, while GS-331007 AUC was 27% higher during co-administration with 3D compared to sofosbuvir alone. Sofosbuvir C_{max} and AUC were 64% and 94% higher, respectively, whereas GS-331007 AUC was 32% higher during co-administration with 2D compared to sofosbuvir alone. The increases in SOF and GS-331007 exposures are not considered clinically significant. No subjects discontinued the study due to study drug-related adverse events.

Conclusions: Ombitasvir/paritaprevir/r +/− dasabuvir increased sofosbuvir plasma concentrations with limited effects (particularly AUC) on GS-331007 plasma concentrations. No dose adjustment is needed for sofosbuvir during co-administration with the 3D or 2D regimen. No dose adjustment is needed for ombitasvir, paritaprevir or dasabuvir when administered as the 3D or 2D regimen with sofosbuvir.

Cohort 1	Period 1 (Days 1 to 14)	Period 2 (Days 15 to 21)
	DAAs	DAAs
		Sofosbuvir
Cohort 2	**Period 1 (Days 1 to 7)**	**Period 2 (Days 8 to 21)**
	Sofosbuvir	DAAs
		Sofosbuvir

Figure 1. Study design.

P0906

INHIBITION OF HCV REPLICATION BY CCR5 BLOCKADE WITH CENICRIVIROC AND MARAVIROC

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Background and Aims: CCR5 is a chemokine co-receptor best known for its central role in HIV binding to T-cells. However, CCR5 is expressed in hepatocytes, stellate cells and Kupffer cells within the liver, and may mediate liver fibrosis. Interaction between HIV (or gp120) and CCR5 has been shown to upregulate HCV replication as well. We hypothesized that CCR5 blockade might modulate HCV replication. We examined the effect of CCR5 blockade using two potent receptor antagonists, cenicriviroc (dual CCR2/5 antagonist) and maraviroc (CCR5 antagonist) on HCV replication.

Methods: Briefly, 100,000 HUH7.5-JFH1 human hepatocyte cells, which constitutively produce a cell-line adapted strain of HCV genotype 2 and express CCR5 were seeded in wells. The next day, cells were incubated with 25 ug/ml, 0.25 ug/ml or 0.0025 ug/ml of the antiviral drugs under investigation. These included maraviroc or cenicriviroc, sofosbuvir (an HCV polymerase inhibitor positive control) or raltegravir (an HIV integrase inhibitor negative control). The culture supernatants were collected after 24 hours of incubation with the drug. Cell number was evaluated to determine if cell viability affected HCV viral load production. HCV replication was monitored using a commercial HCV Core Antigen ELISA assay (Cell BioLabs, San Diego, CA).

Results: A statistically significant and dose-dependent response was observed for maraviroc, cenicriviroc, and sofosbuvir at the 0.25 ug/ml and 25 ug/ml concentrations but not at 0.0025 ug/ml. Raltegravir had no effect on HCV Core Antigen production, nor did DMSO buffer controls. Cell viability was not affected by any drug or by the DMSO buffer solution.

Conclusions: As a result of CCR5 blockade – maraviroc and cenicriviroc – significantly reduced HCV Core Antigen production in an *in vitro* HUH7.5 JFH1 human hepatocyte cell line at levels of inhibition similar to that observed for sofosbuvir, a direct acting HCV RNA polymerase inhibitor. This potent inhibition suggests that CCR5 may represent a new target for multidrug HCV therapy.

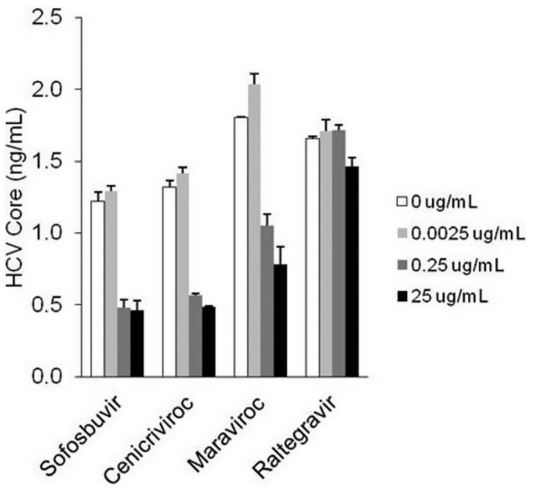

Figure: HCV Core ELISA.

P0907

PHARMACOKINETICS, PHARMACODYNAMICS, AND TOXICITY PROFILE OF RG-101, A NOVEL GalNAc-CONJUGATED HEPATOCYTE-TARGETNG INHIBITOR OF MICRORNA-122, IN RODENTS AND CYNOMOLGUS MONKEYS

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Background and Aims: RG-101 is a novel phosphorothiate (PS) oligonucleotide (oligo) that binds and efficiently inhibits microRNA (miR)-122, which is abundantly and specifically expressed in hepatocytes and plays a key role in the replication of the hepatitis C virus (HCV). Preferential targeting to hepatocytes was achieved by conjugation of the oligo to GalNAc through a phosphodiester linker, which results in a pro-drug that is metabolized in the liver to an active unconjugated oligo. Previous studies using an unconjugated miR-122 anti-miR demonstrated sustained PD activity and no adverse effects in normal mice treated weekly for up to 50 weeks.

Methods: To support first-in-human trials, pharmacokinetics (PK), pharmacodynamics (PD), and toxicity (Tox) of RG-101 administered subcutaneously in mice and cynomolgus monkeys were studied at doses from 0.1 to 450 mg/kg in mice and 1.5 to 150 mg/kg in monkeys.

Results: After injection, rapid absorption into plasma was observed with T_{max} of 0.5–4 hours. RG-101 is stable in plasma and no unconjugated oligo was detected. Uptake in liver and kidney

was rapid, and RG-101 was rapidly and efficiently cleaved to unconjugated oligo in the liver. Half-life of the unconjugated oligo in the liver was 8–14 days in mouse and monkey. Measurement of an endogenous miR-122 target mRNA, *aldoa*, in the liver revealed rapid onset of action with 80% maximal activity within 24 h and greater than 60 days duration of action. Pro-inflammatory effects in mouse and rat were minimal at high doses. In single and repeat dose studies, typical PS oligo class effects, including complement activation and prolonged APTT, were observed in monkeys, but were not considered adverse. No renal toxicity occurred, which reflects the biased distribution of GalNAc-conjugated oligo to the liver. Delayed dose-related increases in liver enzymes, including alanine aminotransferase (ALT), were observed after the first dose with no further increases seen after subsequent doses. None of these findings were associated with adverse clinical signs and complete-to-partial recovery occurred 3 months after last dose. No Observed Adverse Effect Levels (NOAELs) of 450 mg/kg and 45 mg/kg in mouse and monkey, respectively, were declared for RG-101, producing a 30–100 fold therapeutic index.

Conclusions: Taken together, the PK/PD/Tox profile of RG-101 suggests that conjugation of oligos to GalNAc produces significant advantages that are likely to translate to more effective and safer clinical outcomes.

P0908

ADHERENCE TO OMBITASVIR/PARITAPREVIR/R, DASABUVIR, AND RIBAVIRIN IS >98% IN THE SAPPHIRE-I AND SAPPHIRE-II TRIALS

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Background and Aims: Scant data are available on adherence with direct-acting antiviral (DAA) regimens or the impact of poor adherence on virologic outcomes. Ombitasvir is a NS5A inhibitor and dasabuvir is an NS5B RNA polymerase inhibitor. Paritaprevir (formerly ABT-450) is a HCV NS3/4A protease inhibitor identified by AbbVie and Enanta and co-dosed with the pharmacokinetic enhancer ritonavir (r), to improve peak, trough and overall drug concentrations. We report adherence rates in the 3DAA + ribavirin (3D+RBV) and placebo arms in the phase 3 double-blind, placebo-controlled SAPPHIRE trials among 1025 HCV genotype (GT) 1-infected patients in 20 countries.

Methods: Treatment-naïve (n = 631; SAPPHIRE-I) and treatment-experienced patients (n = 394; SAPPHIRE-II) were randomized to 12 weeks of active treatment (3D+RBV: co-formulated ombitasvir/paritaprevir/r [25 mg/150 mg/100 mg QD] and dasabuvir [250 mg BID] with weight-based RBV [1000 or 1200 mg]), or placebo. For all patients with pill count data available during double-blind treatment, adherence was calculated as: (pills dispensed minus pills returned)/(total pills expected to be taken for days on study drug).

Results: Of 1025 patients, 56% were male, 90% were white, mean (SD) age was 50.9 (10.6) years, and mean (SD) BMI was 26.0 (4.2) kg/m^2. Intent-to-treat SVR12 rates were 96.4% (SAPPHIRE-I) and 96.3% (SAPPHIRE-II). Mean adherence to each study drug in both trials was >98.8% for patients on 3D+RBV or placebo (Table). In the 3D+RBV arms, 5 patients had adherence <80% to one or more study drug; none of the 5 experienced virologic failure. In SAPPHIRE-I, patients with on-treatment virologic breakthrough (n = 1; 0.2%) or post-treatment relapse (n = 7; 1.5%) had ≥95% adherence to all 3 study drugs. In SAPPHIRE-II, all patients with post-treatment relapse (n = 7; 2.4%) had >98% adherence to 3D; 5 of 7 had >98% adherence to RBV, and 2 had missing RBV pill count data. In both trials, most adverse events were mild, and those leading to treatment discontinuation were infrequent: 0.8% (6/770.)

Table: Summary of adherence to study drugs during double-blind treatment

	Adherence [a], Mean % (SD), n [b]			
	SAPPHIRE-I HCV GT1 (treatment-naïve)		SAPPHIRE-II HCV GT1 (treatment-experienced [c])	
	3D+RBV	Placebo	3D+RBV	Placebo
Ombitasvir/Paritaprevir/r	99.71 (1.75) n = 444	99.79 (1.81) n = 152	99.81 (1.00) n = 289	99.62 (0.84) n = 92
Dasabuvir	99.22 (3.07) n = 444	99.34 (2.13) n = 152	99.49 (1.10) n = 289	99.25 (0.84) n = 92
RBV	98.85 (3.96) n = 427	98.93 (2.18) n = 152	99.46 (5.66) n = 272	99.25 (2.42) n = 92

[a] Adherence reflects days on study drug for all patients with pill count data available, including patients who discontinued or interrupted study drug for adverse events or other reasons.
[b] Adherence data for each type of pill not available for all patients.
[c] Treatment-experienced patients had a prior relapse, partial, or null response to pegIFN/ribavirin therapy.

Conclusions: In this analysis, the multi-targeted IFN-free regimen of ombitasvir/paritaprevir/r and dasabuvir with RBV was well tolerated, with high rates of adherence (>98%) to all study drugs, including RBV at 1000 or 1200 mg. Low adherence rates were infrequent and did not lead to virologic failure.

P0909

ANALYSIS OF NATURAL RESISTANCE PROFILE TO NS5A REPLICATION COMPLEX INHIBITORS IN DIFFERENT HEPATITIS C GENOTYPES

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Background and Aims: Recent progress in the understanding of hepatitis C virus (HCV) biology has facilitated the development of direct-acting antiviral agents (DAAs) that target specific steps in the viral replication cycle. Several compounds thought to bind directly with NS5A are now in various clinical trial phases, including the most advanced, daclatasvir, ledipasvir and ABT-267. Resistance mutations have been mapped to the N-terminal region of NS5A (the first 100 amino acids within domain 1). Analysis from daclatasvir clinical trials showed that treatment failure was related to pre-existing variants exhibiting resistance to NS5A inhibitors (NS5A-I) in patients infected by genotype (G) 1a and 1b. We aimed to evaluate the frequency of natural resistant variants to NS5A-I in different HCV genotypes.

Methods: Changes reported to be associated with NS5A-I resistance (positions 28, 30, 31, 54, 58, 93) were examined. A total of 2831 (domain 1: aa 1–100) NS5A sequences deposited at Los Alamos HCV database were analysed: 1209 sequences belonged to G1a, 1552 to G1b, 48 to G3a and 22 to G4 (17 subtype 4a and 5 subtype 4d).

Results: Overall, resistance associated mutations (RAM) were detected in 641 of 2831 (22.5%) isolates. In G1a sequences, 117 (10%) RAM has been detected: 37 sequences showed aa change

at position M28, 16 at Q30, 6 at L31, 37 at H58, 7 at Y93 and 14 sequences harboured 2 aa changes at least. In G1b sequences, 519 (33%) RAM has been detected: 78 isolates showed mutation at aa L31, 364 at Q54, 12 at Y93 and 65 strains harboured 2 aa changes at least. None of 48 G3a sequences showed RAM, 2 of 22 (9%) G4 sequences harboured P58T resistance mutation; both these isolates belonged to subtype 4d. The presence of natural RAM was more frequently reported in G1b than in G1a isolates (33% vs 10%; $p < 0.0001$). The frequency of variants harbouring more than one RAM was significantly different between G1a and G1b (1% vs 4%; $p < 0.0001$). Comparison among different genotype showed a significant difference ($p < 0.0001$).

Conclusions: Natural polymorphisms in NS5A at positions that may influence susceptibility to NS5A-I were less frequently observed in genotypes 3a and 4. Surprisingly, G1a isolates showed a lower prevalence of natural RAM respect to G1b, both considering single RAM or variants with more than one resistance mutation. This information may provide further insights in evaluate the right interferon free regimen including DAAs combination.

P0910

NO EVIDENCE OF PHARMACOKINETIC DRUG–DRUG INTERACTION IN HEALTHY SUBJECTS BETWEEN COADMINISTERED GRAZOPREVIR (MK-5172)/ELBASVIR (MK-8742) AND SOFOSBUVIR

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Background and Aims: Grazoprevir (MK-5172) is a potent, once-daily inhibitor of the hepatitis C virus (HCV) NS3/4A protease, and elbasvir (MK-8742) is a potent, once-daily inhibitor of the HCV NS5A replication complex that are being developed as a fixed-dose combination therapy for the treatment of chronic HCV infection. This study evaluated the effect of grazoprevir and elbasvir on the pharmacokinetics of sofosbuvir (SOF), an HCV NS5B inhibitor, when coadministered in healthy subjects.

Methods: This was an open-label, 2-period, fixed-sequence study to assess the effect of multiple oral doses of grazoprevir and elbasvir on the pharmacokinetics of a single oral dose of SOF. Sixteen healthy adult male and female subjects were enrolled. In Period 1, subjects received a single oral 400 mg dose of SOF. Following an 8 day washout period, in Period 2 multiple oral doses of 200 mg grazoprevir and 50 mg elbasvir were co-administered once daily from Days 1 to 15, inclusive. On Day 11, a single oral dose of SOF was co-administered with the dose of grazoprevir and elbasvir. Plasma pharmacokinetic parameters of SOF and its principal nucleoside metabolite (GS-331007) were measured in Period 1 and following Day 11 in Period 2.

Results: SOF + grazoprevir + elbasvir/SOF alone geometric mean ratios (GMRs) [90% confidence intervals (90%CIs)] for plasma SOF $AUC_{0-\infty}$ and C_{max} were 2.43 [2.12, 2.79] and 2.27 [1.72, 2.99], respectively. These changes in SOF are not considered to be clinically meaningful based on the safety margins of SOF. The GMRs [90% CIs] for plasma GS-331007 $AUC_{0-infty;}$ and C_{max} for the same comparison were 1.13 [1.05, 1.21] and 0.87 [0.78, 0.96], respectively. These changes in GS-331007 are not considered to be clinically meaningful. Coadministration of SOF, grazoprevir, and elbasvir was generally well tolerated.

Conclusions: Multiple dose administration of 200 mg grazoprevir and 50 mg elbasvir daily with a single dose of SOF was generally well tolerated by healthy subjects in this study. Co-administration of elbasvir and grazoprevir with SOF had no clinically meaningful effect on the PK of SOF and its metabolite GS-331007. Taken together with the lack of potential for SOF to perpetrate a drug–drug interaction on grazoprevir or elbasvir, these results suggest that SOF, grazoprevir, and elbasvir may be coadministered without dose adjustment.

P0911

CHARACTERIZATION OF CLINICAL PREDICTORS OF NATURALLY OCCURRING NS3/NS4A PROTEASE POLYMORPHISM IN GENOTYPE 1 HEPATITIS C VIRUS INFECTED PATIENTS

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Background and Aims: Chronic hepatitis C is a major cause of liver disease worldwide. Its high replication rate and lack of proofreading activity lead to a great diversity in viral population (polymorphisms) that may emerge as dominant strains in a DAA's therapy set. Spontaneously occurring resistance may impair the rate of success in some macrocyclic NS3 protease inhibitors (PI) based regimens. This study aimed to evaluate potential associations between the frequency of amino acid variations in NS3/NS4A protease of hepatitis C virus (HCV) and demographic/clinical features in chronic HCV infected patients.

Methods: Clinical and epidemiological data were retrieved from medical records of 247 HCV chronically infected patients consecutively evaluated in 5 tertiary centers in Brazil. HCV NS3 analysis was performed by direct sequencing with Sanger-based technology.

Results: 156 subjects (63.1%) were male and the mean age was 44.9(±9.6) years. Sixty-seven individuals (27.1%) had history of ethanol use. One hundred and twelve patients (45.3%) were coinfected with HIV. Overall, 21.9% of analyzed subjects had at least one amino acid substitution in HCV NS3 region [95% CI: 15.0–53.9]. Fourteen HCV samples (5.6%) harbored at least one NS3 PI resistance mutation (T54S, V55A or Q80R) [95% CI: 3.1–9.3]. Variables independently associated with NS3 amino acids substitutions were HCV subtype 1b (OR: 5.68, 95% CI: 2.61–12.35), total bilirubin level >1.5 ULN (OR: 5.25, 95% CI: 1.19–23.16) and albumin level <3.5 g/dL (OR: 3.88, 95% CI: 1.19–23.16).

Conclusions: The frequency of HCV NS3 polymorphism in patients with laboratory markers of advanced liver disease was high, indicating a possible relation between length of hepatopathy and accumulation of such amino acid substitutions. HCV protease in subtype 1b appears to be highly variable in our country.

P0912

EARLY-PHASE HCV KINETICS AND ROLE OF PRE-EXISTING RESISTANCE IN CIRRHOTIC OR INTERFERON-INSENSITIVE PATIENTS ON DACLATASVIR PLUS ASUNAPREVIR

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Background and Aims: The combination of daclatasvir (DCV) and asunaprevir (ASV) obtains HCV clearance in up to 90% of patients infected with HCV Gt-1, but treatment response is slower in subjects with a previous non-response to pegIFN+ribavirin (P/R).

No data are available on early HCV-RNA kinetics during DCV+ASV treatment, nor on pre-existing resistance associated variants (RAVs), for Gt-1 patients with previous P/R failure and/or cirrhosis, in whom treatment failure would have major clinical relevance.

Methods: Nine patients with HCV-1b (8 Child A cirrhosis; 8 previous non responders to P/R: 6 null, 2 relapse) receiving DCV 60 mg qd and ASV 100 mg bid were studied. Baseline (BL) presence of NS3-NS5A RAVs was assessed by population sequencing. HCV-RNA was quantified at BL, early time-points (2 h-6 h-24 h-30 h-48 h-72 h-96 h-1w-2w) and per protocol (4w-8w-12w-20w-24w-28w-32w), and its kinetics modeled (Guedj, PNAS 2012). Sustained viral response (SVR) at w4 are presented.

Results: 6/9 patients had a rapid viral response (RVR), and 8/9 patients achieved SVR_4, with 1 RVR-patient relapsing at w4 of follow-up. In all patients, HCV-RNA decay was biphasic, starting 6 h after initial dosing (median [IQR] HCV-RNA decay = −1.0 [−1.7; −0.8] log IU/ml) with the steepest decline at 24 h (median [IQR] HCV-RNA decay = −2.8 [−2.9; −2.2] log IU/ml) then slowing down. Since onset, non-RVR patients tended to have slower BL-24 h HCV-RNA decay as compared to RVRs (median [IQR] = −2.4 [−2.8; −1.4] *vs* −2.9 [−3.0; −2.9] log IU/ml), resulting in lower mean virion clearance rates (c) (15.8 day-1 *vs* 26.0 day-1, respectively). This trend was observed also during phase II, where non-RVRs had lower mean cell clearance rates (δ) (0.09 day-1 *vs* 0.26 day-1 in RVRs), resulting in suboptimal BL-2w HCV-RNA decay (median [IQR] = −4.3 [−4.7; −3.4] *vs* −5.1 [−6.1; −4.4] in RVRs) and always quantifiable HCV-RNA values at w2. Notably, the NS5A-RAV Q54H was detected at BL in all 3 non-RVR patients, in the only DCV+ASV relapser and in 1 RVR/SVR patient. No BL NS3-RAVs were detected.

Conclusions: In HCV-1b cirrhotic and/or IFN-insensitive subjects, a suboptimal virological response can be already visualized in the first days of DCV+ASV. The presence of NS5A-RAV Q54H was associated with a slower response (or treatment failure) in real-life setting, and would suggest a cautious management of high risk patients treated with NS5A inhibitors, considering the potential need for a third drug (NS5B inhibitor?).

P0913

INTERACTIONS STUDY OF HCV NSB5 POLYMERASE INHIBITORS WITH NSB5 NON-STRUCTURED PROTEINS TOWARD BETTER DESIGN OF NEW INHIBITORS BASED ON MOLECULAR DOCKING AND PHARMACOPHORE METHODS

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Background and Aims: Since targeting the NSB5, which is an RNA dependent RNA polymerase and play a crucial role in synthesis of negative and positive strand copy of RNA genome, showed to be an effective therapy, new inhibitors can be designed if the key residuals affecting the bonds formation between the inhibitors and NSB5 non-structured proteins are being identified. In this work, the first attempt is to derive these key residuals that are in close contact with a potent inhibitor based on idole moiety, and then designing some new inhibitors based on molecular docking and QSAR analyses.

Methods: The inhibition activities and inhibitors were taken from literature (J. Med. Chem. 2012, 55, 754–765), and then, the most inhibitor (shown in Figure 1) was studied in AutoDock 4.2 program to understand its existing interactions with NSB5 polymerase. The protein complexes were selected from protein databank (http://www.rcsb.org) with the PDB ID of 3GOL. The results of docking were then analyzed with LigandScout 3.03 program (Figure 1). After deriving the key features for increasing the inhibition activities, new compounds were designed and their activities were predicted using the derived robust and validated QSAR model.

Results: From docking analyses, it was observed that the taken potent inhibitor displayed several hydrophobic interactions with Met414, Leu384, Tyr415, Tyr448 and Phe193 residuals, and several hydrogen bond interactions with Gly449, Gln446, Thr448, Tyr415 and Ser368. Based on the derived results, some new compounds were designed considering the increasing the hydrophobic functional groups in detected substituents, and then the inhibition activities of newly designed drugs were calculated by GA-MLR model as below:

pIC_{50} = 11.65(±0.8449) − 0.4173(±0.04646)PCR
− 1.392(±0.4287)GATS2e − 0.1281(±0.01746)RDF090m
+ 6.991(±0.5781)H7u.

This model was built based on 38 compounds as training set with high reliability (R^2 = 0.866, Q^2_{LOO} = 0.817, F = 53.46) and then its external prediction ability was validated using 10 compounds as test set. The results for test set (R^2 = 0.802, RMSE = 0.322, CCC = 0.894) indicated that the derived model is reliable and can be used for predicting the pIC_{50} values for NSB5 polymerase inhibitors.

Conclusions: The predictive ability of the proposed model was found to be useful for predicting the inhibitory activity of taken inhibitors. Some compounds as HCV NSB5 polymerase inhibitor were designed and their inhibition activities were predicted by QSAR derived model (Figure 1).

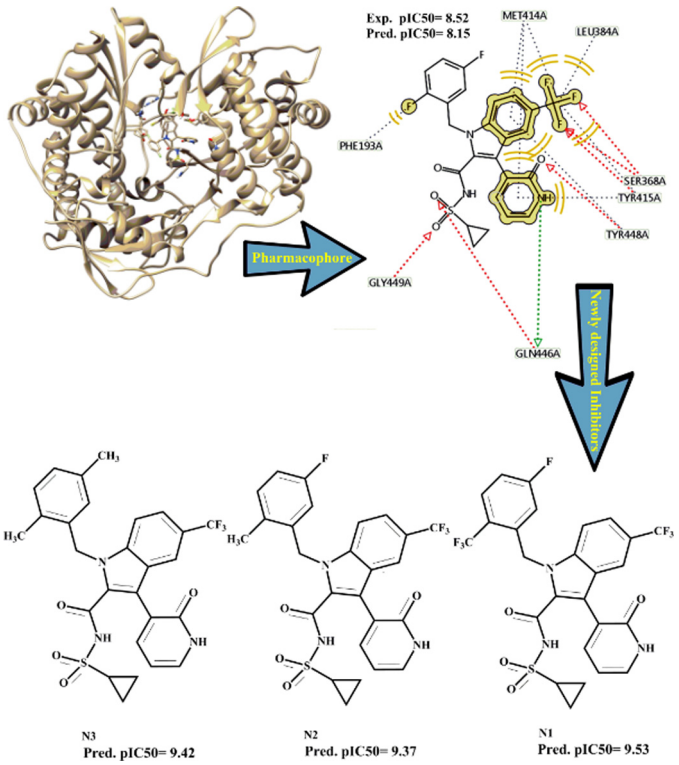

Figure 1.

P0915

CLINICAL RELEVANCE OF BASELINE/EARLY DETECTION AND PERSISTENCE OF RESISTANT ASSOCIATED VARIANTS IN HCV-1 PATIENTS TREATED WITH PROTEASE-INHIBITORS ASSESSED BY ULTRA-DEEP SEQUENCING

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Background and Aims: This study aims to evaluate the clinical utility of ultra-deep pyrosequencing (UDPS) on the detection of minority resistance associated variants (RAVs) in chronic HCV-1 infected patients treated with telaprevir (TVR)/boceprevir (BOC)/simeprevir (SMV) + pegIFN/ribavirin (RBV) at different time points: before, during and after treatment.

Methods: NS3 protease sequences of 55 selected patients (33 virological failures and 22 responders) treated with TVR (N = 43), BOC (N = 10) or SMV (n = 2) + pegIFN/RBV were analyzed. Presence of NS3-RAVs was evaluated by both Sanger-sequencing and UDPS (cutoff = 0.1%;>3000 sequences/patient) at baseline (BL), early time points (8 h–48 h), failure, and after therapy-interruption.

Results: At BL, 13/55 patients (24%) presented NS3-RAVs by both Sanger and UDPS (Q80K = 8; T54S = 3; R155K = 1; V36L+Q80K = 1). Of them, 9 patients experienced virological failure and 4 achieved a sustained virological response (SVR). Additional BL minority RAVs were found only by UDPS, in 6 TVR-treated patients, all with a prevalence <1%. However, their presence was not directly associated with virological failure. Indeed, 2 previous-null-responders with BL minority RAVs (F43S/Q80K) failed TVR triple-therapy with different RAVs (V36M + R155K; V36AM + R155K + T156ST, respectively). Other 4 patients, none previous-null-responders, having BL minority RAVs (D168A/V170A/T54A/Q80K) achieved SVR.

At early time points, additional minority RAVs (no present at BL) were detected in 3/18 (17%) patients, only by UDPS. Among them, 2 previous null-responders failed TVR triple-therapy showing similar mutations at 48 h and at failure (1 HCV1a showed $V36A_{48\,h}$ = 3% + $R155K_{48\,h}$ = 4% and failed with V36M+$R155K_{week8}$ = 100%; 1 HCV1b showed $V36A_{48\,h}$ = 9% +$V36G_{48\,h}$ = 25% +$T54A_{48\,h}$ = 36% and failed with T54AS+$V36A_{week22}$ = 87-1%).

UDPS was also used to characterize RAV persistence in 5 failing patients after at least 48 weeks of therapy interruption: 3/5 still showed persistence. In particular, 2 HCV-1a patients, both failing with V36M+R155K, still showed $R155K_{week100}$ (at 35% and 4%, with a mutational load of 594,022 and 77,966 IU/ml, respectively). Another HCV-1b patient, failing with T54S+A156T, still showed $T54S_{week60}$ (at 3%, with a mutational load of 15,571 IU/ml).

Conclusions: Although UDPS clinical relevance is still poorly understood, it could provide an added value on resistance detection in selected populations: it can help in the identification of patients to high-risk of failure and on monitoring RAVs persistence after PI-discontinuation.

P0916

EMERGENCE OF HEPATITIS C VIRUS GENOTYPE RECOMBINANT FORMS 2K/1B IN GEORGIA

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Background and Aims: Hepatitis C virus (HCV) today is classified into 11 genotypes and many closely related subtypes. Kalinina et al. identified in the early 1990s HCV strains with genetic divergence, which was not due to mutations but to fusions of parts of different HCV genomes. The authors suggested the extension of the current classification with the term "recombinant form (RF)". In 2001, 2k/1b HCV RF was found in St. Petersburg, Russia. Only few cases are identified worldwide. Patients were mainly from Eastern Europe.

The aim of the study was to evaluate the distribution of HCV genotype 2k/1b – recombinant form (St. Petersburg variant) in Georgia.

Methods: We retrospectively analyzed data of HCV genotypes from Medical Center Mrcheveli, Tbilisi, Georgia, 2012–2014 (24 months), in 491 patients with chronic HCV. All samples were sent to laboratory Limbach (Heidelberg, Germany).

Initially all studies were performed with the VERSANT HCV Genotype 2.0, a second generation line probe assay, containing probes targeting the 5′ non coding region (NCR) and for higher resolution of genotype 1 the core regions of the viral genome. All HCV genotypes 2a/2c were further specified by partially gene equencing the NS5A region.

Results: Distribution of genotypes:

Genotype 1 – 198 (40%): Subtypes 1b 189 (95%); 1a 7 (4%); subtype not identified 2 (1%);
Genotype 3a – 155 (31.5%);
Genotype 2 – 136 (28%);
Genotype 4 (non Georgians) – 2 (0.5%).

All HCV genotypes 2 (136) were further specified. 91 (67%) appeared to be recombinant genotypes 2k/1b.

Distribution of RF 2k/1b genotypes by sex: M 81 (89%), F 10 (11%); distribution of RF 2k/1b genotypes by age: mean 44 (18–66); <20 years – 1 (1%), 20–40 years – 35 (38%), 40–60 years – 54 (59%), >60 years – 2 (2%).

Table (abstract P0916).

Authors	Percentage of HCV RF	Country	Journal
Samokhvalov et al.	0.8%	Russia	Voprosy Virusologii 2013 Jan Feb; 58(1): 36–40.
Viazov S et al.	1.2%	Germany	Journal of Medical Virology 2010 Feb; 82(2): 232–238.
Demetriou et al.	1.6%	Cyprus	Journal of Medical Virology 2009 Feb; 81(2): 238–248.
Kurbanov F et al.	2% Russia; 1 (1%) in Uzbekistan	7 countries	The Journal of Infectious Diseases 2008; 198(10): 1448–1456.
Kurbanov F et al.	1.8%	Uzbekistan	Hepatology Research 2008 May; 38(5): 457–464.
Tallo T et al.	0.5%	Estonia	Journal of Medical Virology 2007 Apr; 79(4): 374–382.

Conclusions: We can conclude that the HCV genotype RF 2k/1b is common in Georgia. About 18.5% of all genotypes and 67% of genotype 2 belonged to a recombinant HCV form: 5′ NCR genotype 2 and NS5 region genotype 1b.
Based on the present literature the prevalence of recombinant forms in different countries do not exceed 2% among of all HCV genotypes.
Because HCV genotypes are predictors of outcome of interferon based therapy (until now most important in Georgia), the widespread of these genotypes in our country can change the HCV treatment options.
New anti-HCV drugs will replaced interferon therapy and will not be dependent on the genotypes. Therefore, identification of resistance to new generation anti-HCV drugs may be a diagnostic challenge.

P1352

PHARMACOKINETICS AND PHARMACOLOGY OF RG-101, A NOVEL GalNAc-CONJUGATED OLIGONUCLEOTIDE TARGETING MICRORNA-122, IN HEALTHY VOLUNTEERS

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Background and Aims: RG-101 is a GalNAc-conjugated chemically modified oligonucleotide designed to inhibit microRNA-122 (the most prevalent microRNA in hepatocytes) and inhibit the replication of HCV. Prior studies showed enhanced delivery to hepatocytes and increased potency of RG-101 relative to a non-conjugated oligonucleotide.
Methods: RG-101 is being evaluated in a four part Phase I clinical study: part A was a single subcutaneous (SC) ascending dose study in healthy volunteers (HV) with doses of 0.5, 1, 2, 4, and 8 mg/kg; part B was a repeat dose study in HV (4 doses of 2 mg/kg given once every 28 days); part C was a drug–drug interaction (DDI) study with single dose RG-101 (2 mg/kg) added on to simeprevir in HV; and part D included a single dose study of 2 and 4 mg/kg RG-101 in HCV genotype 1, 3, and 4 chronic hepatitis C (CHC) patients (data not shown).
Results: RG-101 was well tolerated in HV at all doses with no significant adverse events identified to date. The C_{max} of RG-101 ranged from 0.03 μg/ml (at 0.5 mg/kg) to 8.8 μg/ml (at 8 mg/kg) and the AUC_{0-24hr} ranged from 0.1 μg·hr/ml (at 0.5 mg/kg) to 85.9 μg·hr/ml (at 8 mg/kg). RG-101 increased serum alkaline phosphatase (ALP) (a direct miR-122 target) and decreased serum cholesterol (CHOL) (an indirect miR-122 target). Maximum changes in ALP and CHOL after a single dose of RG-101 were observed at 2 mg/kg when the C_{max} was 1.4 μg/ml and the AUC_{0-24hr} was 6.2 μg·hr/ml. In HV the time to max increase in ALP was ~14 days and time to max reduction in CHOL was ~21 days. Neither ALP nor CHOL returned to baseline by Day 57. RG-101 was also evaluated in combination with simeprevir. HV were administered 150 mg simeprevir on day 1, then daily on days 5 through 14. A single dose of RG-101 (2 mg/kg) was co-administered on day 5. The C_{max} (1.5 μg/ml) and AUC_{0-24hr} (8.4 μg·hr/ml) of RG-101 was not changed by addition of simeprevir. RG-101 had no effect on simeprevir PK parameters with the C_{max} being 1.5 μg/ml and 1.4 μg/ml and AUC_{0-24hr} being 15.5 μg·hr/ml and 14.8 μg·hr/ml in the absence or presence of RG-101 respectively. The combination of RG-101 and simeprevir was well tolerated and no significant adverse events were observed.
Conclusions: RG-101 alone or in combination with simeprevir was well tolerated in HV. No DDI between RG-101 and simeprevir was observed. Maximum pharmacological activity, as measured by increased ALP and decreased CHOL, was observed with RG-101 in HV at 2–4 μg·mg/kg.

Fatty liver disease: a. Experimental

P0917

ROLE OF FXR CONTROLLED Chop IN MEDIATING BILE ACID EFFECTS IN NAFLD

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Background and Aims: Farnesoid-X-receptor (FXR) has an important regulatory function in hepatic bile acid (BA) and lipid homeostasis. We aimed to explore the role of FXR and BAs in the control of Chop (recently suggested to impact on lipid homeostasis via regulation of C/ebpa) as potential regulators of hepatic lipid metabolism and inflammation.
Methods: Wild type (WT) and FXR knock-out (KO) mice were fed a MCD diet to induce NASH. Hepatic gene-expression was profiled by RT-QPCR for markers of hepatic lipid metabolism and inflammation. Moreover, serum biochemistry, liver histology and hepatic TG content were assessed. HepG2 cells were treated with glucose, with/without FXR and RXR agonists. Gene-expression was analysed by RT-QPCR and chromatin-immuno-precipitation was performed.
Results: MCD feeding resulted in increased serum BA levels and liver enzymes in both WT and FXR KO mice. However, Chop mRNA was up-regulated only in MCD fed WT but not in FXR KO mice. Moreover, mRNA levels of C/ebpa were down-regulated in MCD challenged WT, but not FXR KO mice. WT MCD mice display markedly increased VLDL-receptor mRNA levels consistent with elevated hepatic TG content. Hepatic inflammation in response to MCD (reflected by F4/80, TNFa mRNA expression) was aggravated by absence of FXR. High BA and low glucose levels increased Chop and subsequently repressed C/ebpa expression in a FXR/RXR dependent fashion in HepG2 cells. Finally, a FXR/RXR binding site was identified in the human promoter of Chop demonstrating a highly conserved regulated pathway.
Conclusions: These findings demonstrate that glucose and BAs control Chop expression via FXR/RXR therefore providing novel insights into pathogenesis and treatment of NAFLD.
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P0918

IMPAIRED PPARα SIGNALLING INFLUENCES BILE ACID HOMEOSTASIS AND INFLAMMATION IN ATGL LIVER KO MICE

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Background and Aims: Adipose triglyceride lipase (ATGL) is the main enzyme in intracellular lipolysis and generates PPARα ligands, such as linoleic acid. PPARα activation represses the rate limiting enzyme in bile acid (BA) synthesis Cyp7a1, indicating a key role of PPARα in biliary homeostasis. Therefore, we aimed to determine

the effects of hepatic ATGL deficiency (and subsequently impaired fatty acid signalling via PPARα) on BA homeostasis.
Methods: Bile flow was analyzed in liver specific ATGL knock out (ATGL LKO) and wild type (WT) littermates. Furthermore these mice were subjected to bile duct ligation (BDL) for 7days (acute cholestasis model). RT-QPCR was used to assess BA synthesis, inflammation and fibrosis gene expression markers. Serum biochemistry and histology were also assessed.
Results: ATGL LKO mice show increased bile flow ($p < 0.05$) compared to WT mice. In line, mRNA levels of Cyp7a1 were 4fold induced ($p < 0.05$). Inflammatory markers F4/80 and iNOs were increased 2.2-fold ($p < 0.05$) and 7fold ($p < 0.05$), in line with impaired PPARα signalling. Importantly, acute cholestasis in ATGL LKO mice increased F4/80 expression about 10-fold vs. WT BDL mice ($p < 0.05$). Additionally, mRNA expression of fibrotic marker TGF-β ($p = 0.087$) and elevated proliferation of cholangiocytes reflected by induced CK19 levels ($p = 0.057$) tended to be increased. Of note, mRNA levels of key hepatobiliary (basolateral and apical) BA transporters and levels of serum liver enzymes, cholesterol, TG and BA levels did not differ between groups.
Conclusions: Loss of PPARα activity due to impaired hepatic fatty acid signalling influences BA homeostasis and predisposes to cholestasis-induced inflammation and fibrosis, therefore ATGL and/or PPARα represent potential therapeutic targets.

P0919
DEFECTIVE THERMOGENIC ADAPTATION TO HIGH CALORIE INTAKE: A CONTENDER IN NASH PATHOGENESIS IN FOZ/FOZ MICE

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Background and Aims: Foz/foz mice fed a High Fat Diet (HFD) represent an appropriate model to study NASH in the context of metabolic syndrome. Foz/foz mice carry a spontaneous mutation in Alms1 encoding for a protein of the primary cilium of yet unknown function. The aim of the study is to understand mechanism contributing to their obese, metabolic and liver phenotype.
Methods: Foz/foz mice are hyperphagic. Pair feeding experiment in which foz/foz mice have restricted access to HFD to the exact equal amount of their WT littermate was set up. Activity level, oxygen consumption, thermogenic adaptation was evaluated.
Results: Compared to WT, 4 weeks of HFD induced more severe obesity, insulin resistance, steatosis and liver and adipose inflammation in foz/foz mice. Caloric restriction to foz/foz mice improved but did not restore metabolic disturbances. Physical activity, circadian rhythm, basal metabolism and body temperature were unaffected in foz/foz mice. By contrast, thermogenic adaptation of their brown adipose tissue (BAT) was impaired. In Wt mice, HFD reduced BAT fat load, increased mtDNA content and up-regulated UCP-1, PGC1α and DIO2 consistent with adaptive fat burning. By contrast in foz/foz mice HFD markedly increased BAT lipid accumulation, up regulation of mtDNA content and thermogenic genes was lower or blunted compared to HFD-fed WT mice. Upon exposure to 4°C for 6 hours, body temperature fell by 2.1°C in WT mice and by 3.9°C in foz/foz mice ($p < 0.05$) and up-regulation of thermogenic genes was 40 to 60% lower in HFD-fed foz/foz compared WT mice. Thermogenic failure was further confirmed by a significant lower up-take of ^{18}FDG (PET-Scan) by foz/foz BAT than by WT BAT upon cold exposure.
Conclusions: Overall, these results suggest that in this model reduced adaptive thermogenesis contributes to obesity and insulin resistance, eventually driving NASH and call upon evaluating targeting of BAT for therapeutic intervention.

P0920
SPECIFIC MODULATION OF HEPATIC ESTROGEN RECEPTOR ALPHA PREVENTS FATTY LIVER BUT ALSO OBESITY AND INSULIN RESISTANCE INDUCED BY A HIGH FAT DIET IN MICE

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Background and Aims: Recent experimental data suggest that hepatic Estrogen Receptor alpha (ERα) activation can prevent fatty liver diseases. Estrogens modulate gene expression through the stimulation of the two distinct transactivation functions AF1 and AF2 harbored by ERα. To further characterize the therapeutic potential of ERα modulation, we questioned here the respective roles of (i) hepatic ERα and (ii) ERαAF1 in the protective effects against the liver and systemic metabolic disorders induced by a high-fat diet (HFD).
Methods: Female mice deleted for the entire ERα (ERα°), only for ERαAF1 (ERα-AF1°) or selectively for the hepatic ERα (ERαLiv°), and their respective wild-type littermates (WT) were used. Four-week-old mice were ovariectomized then fed with a HFD (45% fat) and treated or not with pellets delivering Tamoxifen [1.2 mg/kg/d; subcutaneous, a Selective Estrogen Receptor Modulator (SERM) used as a pharmacological tool, known to specifically activate ERαAF1] for 12 weeks.
Results: In WT mice, TMX strongly prevents HFD-induced body weight gain, adipose tissue accumulation, insulin resistance and fatty liver (histological analyses and triglycerides concentration) ($p < 0.01$ for all parameters). Beneficial effects of TMX are totally abrogated in ERα° and ERα-AF1° confirming that ERα-AF1 activation is absolutely required. In addition, the protective effects on fatty liver are abrogated in ERαLiver° mice. More surprisingly, beneficial effects of TMX on HFD-induced obesity and insulin resistance are also totally abrogated in ERαLiver° mice.
Conclusions: Beyond the protective effects on fatty liver diseases, selective modulation (AF1) of hepatic ERα appears sufficient to prevent obesity and impaired glucose homeostasis induced by a HFD, and should be considered as a promising therapeutic target for the prevention of metabolic syndrome and NAFLD.

P0921
MALE PREVALENCE AND FEATURES OF ADVANCED NASH WITH CIRRHOSIS IN THE LIVER KNOCKOUT OF CYP51 FROM CHOLESTEROL SYNTHESIS

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Background and Aims: Accumulation of free cholesterol in the liver promotes hepatocyte apoptosis and fibrosis, and is associated with NASH pathogenesis. Lanosterol 14α-demethylase (CYP51) presents a rate-limiting step in the latter part of cholesterol synthesis. Applying the liver conditional knockout of *Cyp51* (LKO),

we show that blocking hepatic cholesterol synthesis in adult mice causes NASH-like liver changes (Lorbek et al., in review). Herein we describe the male predominant most severe phenotypes of the *Cyp51* LKO mice that stopped their development prior to adulthood.
Methods: From 376 mice (WT and LKO) that survived to the weaning period, 19 (4 female, 15 male) were underdeveloped, with jaundice and hepatomegaly (runts) and were euthanized at 5–10 weeks of age. All runts were of the LKO genotype. After initial histochemical evaluation and analysis of sterol metabolites we applied expression profiling by Affymetrix microarrays to search for molecular signatures that would differentiate runts from the rest of *Cyp51* LKOs.
Results: The runt livers were severely damaged, resembling NASH with cirrhosis, with frequent apoptoses and mitoses, though without the accumulation of lipids. Sirius red staining showed bridging fibrosis of the portal areas. This was accompanied by moderate to severe oval cell response and immune cell infiltration. Microarray analysis of runts compared to age-matched non-cachectic LKOs gave 5983 differentially expressed genes. Among significantly elevated genes in runts are *Spp1* (osteopontin), *Cxcl10* and c-*Jun*, all linked to NASH pathogenesis. Gene set enrichment analysis of KEGG pathways showed enrichment of TGFβ signalling (involved in apoptotic and fibrotic processes), apoptosis, signalling with growth factors (VEGF, PDGF, ErbB) and cancer related pathways. Gene ontology term enrichment listed the unfolded protein response (integrated stress response) as significantly deregulated, which is in line with our in-house hypothesis that changes in cholesterol balance result in ER stress. On the metabolite level, runts compared to non-cahectic LKOs exhibit further elevation in hepatic lanosterol and 24,25-dihydrolanosterol, both substrates of CYP51, and significantly elevated free cholesterol.
Conclusions: We present a novel model where disrupted cholesterol synthesis results in NASH-like symptoms without the presence of steatosis. The severity of liver damage depends on the cholesterol balance. The male-predominance in the most severe *Cyp51* LKO phenotype is under investigation.

P0922
THE COMBINATION OF PROBIOTICS AND PREBIOTICS SUPPLEMENTATION IMPROVES LIPID METABOLISM, NAFLD AND OBESITY IN OB/OB MICE

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Background and Aims: Recent evidence suggests that changes in gut microbiota could modulate the intestinal flora and improve metabolic-related disorders, including nonalcoholic fatty liver disease (NAFLD). The reduction of pro-inflammatory cytokines, the improvement of the immune system, reduction of intestinal infections, could increase lean mass and decrease fat mass. The aim of this study was to examine supplementation with the combination of probiotics and prebiotics in NAFLD and obesity in ob/ob mice.
Methods: Ob/ob male mice, weighing 40 g, received in drinking water a combination of probiotics and prebiotics (*L. acidophilus, L. rhamnosus, L. paracasei, B. lactis* and fructooligosaccharide) for 8 weeks [PRO/PRE (treated group; n = 8). Control group (n = 6) received only drinking water. All animals received standard diet. After 8 weeks, the difference between the initial and final body weight was calculated in all animals and liver tissues were collected for mRNA and miRNA isolation, and histological analysis. Genes related to lipids metabolism (SREBP1C, miR-33a, MTP, PPAR-g) and mitochondrial oxidation (CPT, PPAR-α) were evaluated by RT-qPCR.
Results: The combination of probiotics and prebiotics supplementation improved liver histology, decreased visceral fat mass and weight gain, reducing obesity in comparison to control group. Our study showed a statistically significant decrease in MTP ($p = 0.001$), PPAR-g ($p = 0.023$), PPAR-α ($p = 0.016$) and CPT ($p = 0.010$) mRNA expression and increased expression of miR-33a (0.5 fold change) in treated group compared to control. However, there were no relevant modifications in SREBP1C.
Conclusions: The combination of probiotics and prebiotics supplementation improves liver histology, decreased visceral fat mass and reduced obesity. Besides, this combination of probiotics and prebiotics modulated genes involved in the synthesis, exportation and oxidation of hepatic lipids. However, we can not rule out that other mechanisms such as inflammatory pathways could contribute to improvement of NAFLD.

P0924
CYCLOOXYGENASE-2 REGULATES MIRNA EXPRESSION IN LIVER CELLS THROUGH DEAD BOX HELICASE P68 (DDX5). ROLE IN INSULIN SIGNALING

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Background and Aims: Our previous results revealed that Cyclooxygenase 2 (COX-2) expression is regulated by miRNAs. However, the role of COX-2 in miRNA regulation remains unknown. In the present work, we have studied whether COX-2 regulates the expression profile of miRNAs in liver, and examined the targets of these miRNAs together with their physiopathological properties.
Methods: An RT^{2}miRNA PCR array was performed with microRNA isolated from liver extracts of Wt and transgenic mice overexpressed human COX-2 in hepatocytes. We attempted to confirm the array data in different cell models: in isolated hepatocytes from mice models and in human liver cells stably transfected with a COX-2 expression vector. Western blot analyses were carried out to analyze proteins involved in the biosynthesis of miRNAs with or without treatment with pharmacological inhibitors of different signaling pathways. To evaluate the role of COX-2 in insulin signaling, cells were treated with palmitate to induce insulin resistance. To confirm the results the cells were transfected with DDX5 or COX-2 siRNAs or vector expressing specific miRNAs. Finally, the relationship between COX-2 and miRNA regulation was also studied in human biopsy samples of non-alcoholic steatosis (NAS).
Results: COX-2 represses the expression of miR-23b, miR-146b and miR-183 in liver cells by upregulating the DEAD-box helicase p68 (DDX5) expression through phosphatidylinositol (PI) 3-kinase (PI3K) signaling pathway. Furthermore, COX-2 was shown to inhibit miRNA maturation by associating with the Drosha complex through DDX5 and preventing the conversion of primiRNAs into pre-miRNAs. Analysis of pathways and networks for these miRNAs using the DAVID platform identified the insulin signaling pathway as target. Our results showed that the down-regulation of miRNAs by

COX-2 enhances insulin signaling. Finally, the relationship between COX-2 and the miRNAs was confirmed in NAS.
Conclusions: COX-2 represses the expression of miRNAs implicated in the insulin signaling pathway via a PI3K/p300-dependent upregulation of DDX5, and by modulating the activity of the Drosha complex. Our study proposes a novel miRNA-dependent mechanism through which COX-2 promotes insulin signaling in liver cells.

P0925
PHARMACOLOGICAL EVALUATION OF PIOGLITAZONE AND CANDESARTAN CILEXETIL IN A NOVEL MOUSE MODEL OF NONALCOHOLIC STEATOHEPATITIS, MODIFIED CHOLINE-DEFICIENT, AMINO ACID-DEFINED DIET FED LDL RECEPTOR KNOCKOUT MICE

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Background and Aims: Low-density lipoprotein receptor knockout (LDLR-KO) mouse fed with modified choline-deficient and amino acid-defined (mCDAA) diet exhibits non-alcoholic steatohepatitis (NASH)-like pathophysiology. In order to pharmacologically benchmark this model, effects of pioglitazone, a thiazolidinedione and candesartan cilexetil, an angiotensin II type 1 receptor blocker (ARB) on steatosis and liver fibrosis were examined.
Methods: Pioglitazone (10 mg/kg) and candesartan cilexetil (3 mg/kg) were orally administered once daily to LDLR-KO mice under mCDAA diet for 1 week. After 7 weeks of treatment, blood biochemistry and hepatic histology were assessed, and hepatic expression levels of mRNA, and hepatic triglyceride content were measured.
Results: Pioglitazone suppressed hepatic gene expression of collagen-1 by 43% and attenuated hepatic fibrosis areas by 49%. Pioglitazone also decreased plasma alanine aminotransferase (ALT) levels, liver weight, hepatic triglyceride content and hepatic expression of other fibrosis-related genes such as transforming growth factor β, osteopontin, and tissue inhibitor of metalloproteinase 1, and interleukin-6. Candesartan cilexetil suppressed hepatic collagen-1 gene expression by 33%, while hepatic fibrosis was not affected histomorphometrically. In addition, candesartan cilexetil had no effect on plasma ALT levels, liver weight, hepatic triglyceride content or the hepatic expression of other fibrosis-related genes.
Conclusions: Pioglitazone exhibited anti-fibrotic effects accompanied by anti-steatotic and anti-inflammatory effects, but the effect of candesartan cilexetil was only limited unlike previous reports on ARBs. Since the pharmacological effects of pioglitazone in the current animal model are similar to those reported in patients with NASH, this model may represent some aspects of the pathophysiology of NASH. The partial efficacy of candesartan cilexetil might be attributed to the lesser contribution of the local renin-angiotensin system associated with obesity in this model. Further profiling using other agents or mechanisms that have been tested in the clinic will better clarify the utility of the animal model.

P0926
REPRESENTATION OF HUMAN NON-ALCOHOLIC FATTY LIVER DISEASE IN MURINE MODELS

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder in industrialized countries. Murine models are a central mechanistic platform to study the disease.
Methods: We systematically evaluated genome-wide mRNA expression data from five common murine models of NAFLD [high fat diet (HFD) w/wt fructose, methionine-choline deficient diet, streptozotocin and HFD, PTEN (Fox) KO] in comparison to human data from 77 NAFLD patients and 39 healthy controls spanning the range of disease severity.
Results: We found profound differences between the human and murine transcriptome that override any disease or phenotype signature. Thus of the significantly regulated human genes in NAFLD only between 1 and 16are significantly and concordantly regulated in mice. The dataset, depending on mouse model and human phenotype, shows correct reflection of key pathways, lack of regulation, or reverse effects thereby providing a guide for murine model selection in future experiments to improve applicability to human NAFLD.
Conclusions: In a global cross-species analysis we found profound differences between human and murine expression patterns in fatty liver disease that override any disease or phenotype signature.

P0928
DELETION OF TXNIP PROTECTS AGAINST HFD--INDUCED STEATOHEPATITIS AND LIVER INJURY

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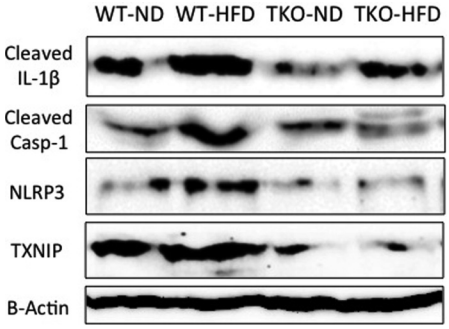
Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of the metabolic syndrome. Oxidative stress and inflammation play a pivotal role in the pathogenesis of NAFLD. Inflammasomes are shown to be implicated in liver damage, steatosis, inflammation and fibrosis. Nevertheless, TXNIP-mediated activation of NLRP3 inflammasome and its involvement in the pathogenesis of HFD-induced NAFLD and NASH remains unknown. In the current study we investigated the role of Thioredoxin-interacting protein TXNIP in steatohepatitis and fibrosis.
Methods: All metabolic parameters including glucose intolerance and plasma levels of total cholesterol and triglycerides were performed after 8-weeks. Using western blotting we examined the changes in the protein levels of TXNIP, NLRP3, caspase-1, IL1β and TNF-α in liver samples from WTND, WTHFD, TKOND and TKOHFD

groups. Furthermore, we tested the impact of TXINB deletion in the developing of steatohepatitis and fibrosis by different histological and immnohistopathological methods including anti-interleukin1 beta (anti-IL1β antibody) and anti-alpha smooth muscle actin (Anti-αSMA) antibody immunostain.
Results: Our results show, massive hepatic steatosis prominent lobular and portal inflammation were more in WTHFD group. Furthermore, significant increase in area and area % of fibrosis by approximately 27 and 40 folds respectively was observed in specimen isolated from WT groups with HFD in comparison to the normal control group (WTND and TKOND) ($n = 5–8$; $p < 0.05$). On the other hand, retraction of fibrosis and little amount fibrous tissue around central vein and portal tract with no fibrous tissue in sinusoidal wall was detected in liver specimen from TKOHFD for 8 weeks ($n = 5–8$; $p < 0.05$). Such observed reduction in area and area% of fibrosis and fibrosis retraction with reduction of accumulation of fibrous tissue in the liver with TKOHFD is consistent with the previous observed reduction in the expression of IL1b, NLRP3, Caspase-1and TXNIP WT liver samples which supporting the important effect of deletion of TXNIP on accumulation of ECM and inhibition of progression of liver fibrosis.
Conclusions: Our results showed that TXNIP deletion can protect against HFD-induced steatohepatitis, and its associated pro-inflammatory and fibrotic response through the activation of the NLRP3 inflammasome and its downstream proinflammatory cytokine IL-1β. Therefore, TXNIP signaling system is a potential therapeutic target to treat NAFLD.



P0929

A DIET-INDUCED MOUSE MODEL OF NASH THAT PRODUCES ROBUST INFLAMMATION AND FIBROSIS

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Background and Aims: Better mouse models that reflect all features of human NASH are needed. We developed a NASH model using a diet which rich in transfats, carbohydrates, and cholesterol (nutritional stress diet, NSD) that mimicks major features of human fibrotic NASH.
Methods: Female 6 wk old C57BL/6 mice ($n = 10$ per group) were fed a control diet (13% kcal fat; 65% kcal carbohydrates), or a high saturated fat diet enhanced in fructose and cholesterol (nutritional stress diet, NSD: 59kJ% kcal lipid, hydrogenated coconut oil; 26kJ% kcal carbohydrates, fructose and glucose; 15kJ% kcal protein, casein; 2% cholesterol), plus fructose-sucrose in drinking water (12.6%: 55:45) for 30 weeks. At sacrifice, liver function, lipid accumulation, IR, inflammation, fibrosis, oxidative stress, and mRNA levels related to fat metabolism, inflammation, fibrogenesis, fibrolysis, and macrophage polarization were analyzed by IHC and qRT-PCR.
Results: Compared to the control mice, mice on the NSD developed a significant increase in body and liver weight, fat deposition, insulin resistance, steatosis, hepatocyte ballooning, and inflammation (NAS score 4), serum parameters of liver injury, and fibrosis (Ishak score 3). Their livers showed significantly increased transcript levels related to fat metabolism, inflammation, M1/M2 polarization, fibrogenesis, and fibrolysis (PPARG, LPL, TNFA, CCL5, MCP1, COL1A1, ACTA2, TGFB1, ITGB6, PDGFRB, PAI1, SPP1, TIMP1, MMP2, MMP8, MMP13). The mRNA levels of IL6 and TNFA were also significantly upregulated in visceral fat tissue. As assessed by IHC, the oxidative stress markers 4-hydroxynonenal and 8-hydroxyguanosine were highly overexpressed in livers of mice fed the NSD.
Conclusions: We describe an optimized diet-induced mouse model that produces all features of human NASH, with robust inflammation and fibrosis. This model will permit to test novel agents that address the major pathologies of human NASH.

P0930

LESSONS FROM HEPATOCYTE-SPECIFIC CYP51 KNOCKOUT MICE: IMPAIRED CHOLESTEROL SYNTHESIS LEADS TO NASH-LIKE OVAL CELL DRIVEN LIVER INJURY

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Background and Aims: Steatosis and NASH might represent separate conditions with potentially different molecular causes. While hepatic triglycerides are a hallmark of steatosis, the role of cholesterol remains controversial. We addressed the role of hepatic cholesterol in liver disease aethiology by applying the liver conditional knockout (LKO) mice of *Cyp51* from cholesterol synthesis.
Methods: 3-week mice were assigned to either standard diet (low-fat no-cholesterol, LFnC) or high-fat diet without (HFnC) or with cholesterol (HFC) for 16 weeks. Blood, bile and organs were collected. Hematoxylin and eosin, Sirius red, pan-cytokeratin stainings, Sudan III and CYP51 stainings were performed on liver sections. Lipids, ALT, AST, corticosterone and TNF-α were measured in plasma. Liver gene expression was performed by qPCR and Affymetrix microarrays and proteins evaluated by western blots. Liver sterols and gallbladder bile acids s were analyzed by GC-MS. Linear regression modeling and empirical Bayes smoothing were applied with the genotype, sex and diet as predictor variables.
Results: LKO mice developed hepatomegaly with oval cell proliferation, fibrosis and inflammation, but without steatosis. The key trigger were reduced cholesterol esters that provoked cell cycle arrest, senescence-associated secretory phenotype and ultimately the oval cell response, while elevated CYP51 substrates promoted the integrated stress response. In spite of the oval cell-driven fibrosis being histologically similar in both sexes, microarray data indicated a female-biased down-regulation of primary metabolism pathways and a stronger immune response in males. Liver injury was ameliorated by dietary fats predominantly in females, whereas dietary cholesterol rectified fibrosis in both sexes. Steatosis provoked by HFC diet did not worsen the liver injury in male LKOs and had beneficial effects in the female LKOs. This is in line with the recent observation that people with hepatic

triglyceride accumulation ("good fat storers") are protected from NASH.
Conclusions: The diminished hepatic cholesterol synthesis through sterol imbalance and ISR led to NASH-features, while dietary fats (in the absence of dietary cholesterol and hepatic cholesterol synthesis) led to steatosis. Only when cholesterol was added to the diet, the NASH-like features of LKOs were ameliorated, placing defective cholesterol synthesis in the focus of NASH.

P0931
OSTEOPONTIN PLAYS A KEY ROLE IN ALCOHOL AND HIGH FAT DIET INDUCED LIVER INJURY

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Background and Aims: Alcoholic Liver Disease (ALD) and Non-Alcoholic Fatty Liver Disease (NAFLD) are highly prevalent liver diseases that may coexist and contribute significantly to liver disease related mortality. Recent literature suggests both disorders share some common pathogenic pathways, including Osteopontin (OPN). There has been little research investigating the combined effects of high fat diet (HFD) and alcohol in liver injury. The current study aimed to investigate role of OPN in a mouse model of alcohol+HFD induced steatohepatitis.
Methods: C57BL6 wt male mice were fed chow or 45% kCal HFD for 12 weeks. Half the mice received a physiologically relevant dose of alcohol (2 g/kg body weight) twice a week. Control mice were given an equal volume of saline. A subset of mice in each treatment group was also given ip injections weekly either with OPN-R3 or control aptamer. Animal weight gain was recorded over time. Blood and livers were harvested at the end of the experiment. The extent of liver injury was examined by histopathology, quantitative analysis of hepatic inflammatory cells, liver and serum biochemistry. Changes in expression of lipid metabolism, inflammation and fibrogenesis related molecules were tested using Q-PCR and immunofluorescence staining.
Results: Superimposing alcohol on the HFD exacerbated the extent of liver damage caused by either agent alone, including increased liver weight, TG, HDL, CHL, serum insulin, lipogenesis (H&E, ORO, SREBP1, SCD1, ACOX1, PPARa), inflammation (leukocytes, Kupffer cells, monocyte) and fibrogenesis (Col1, hepatic stellate cell activation, TGFb). OPN-R3 aptamer intervention significantly reduced myeloid, neutrophil and monocyte cell populations induced by alcohol and HFD in the liver but increased KCs, and lipid deposits, compared to control intervention, suggesting OPN may be required for migration of monocytes and granulocytes from the general circulation to liver tissue, reducing the inflammatory component in steatohepatitis. These observations were confirmed in OPN knockout animals.
Conclusions: OPN is a key mediator of liver injury and has a therapeutic potential for ALD and NAFLD.

P0932
TREATMENT WITH NGM282 SIGNIFICANTLY IMPROVES LIVER HISTOPATHOLOGY IN A MOUSE MODEL OF NON-ALCOHOLIC STEATOHEPATITIS (NASH)

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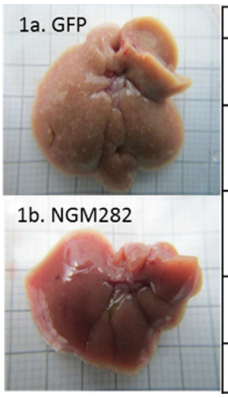
Background and Aims: Human fibroblast growth factor 19 (FGF19) has been shown to regulate glucose homeostasis, body weight, triglycerides synthesis and bile acid (BA) synthesis, factors closely associated with NASH pathogenesis. NGM282 is a novel non-tumorigenic recombinant variant of FGF19 with potent metabolic and BA regulatory activity. The therapeutic potential of NGM282 in NASH was evaluated in a mouse model of steatohepatitis (STAM™ mice).
Methods: STAM™ mice were induced with a single subcutaneous injection of streptozotocin to 2-day old C57BL/6J male mice and a high fat diet [32% (w/w)] feeding beginning at 4 weeks of age. The model is characterized by steatosis, lobular inflammation and hepatocyte ballooning consistent with NASH. Adeno-associated virus encoding green fluorescent protein (GFP) as control or NGM282 were injected intravenously into mice *via* tail vein at 6 weeks of age. Mice were euthanized at 9 weeks of age after the 3 week treatment period. Blood and liver tissue were collected for serum chemistry and histologic analyses.
Results: NGM282 treated mice had significant decreases in total body weight, liver weight and liver-to-body weight ratio reflective of a decrease in total liver fat content (Figure 1a,1b). NGM282 significantly improved all components of the NAFLD activity score (steatosis, lobular inflammation, hepatocyte ballooning) as well as the total score compared to GFP treated controls (Figure 1c). Decreases in ALT, glucose and triglycerides were seen in NGM282 treated mice compared to controls.
Conclusions: NGM282 significantly improved liver histopathology associated with NASH, further supporting the therapeutic potential of this novel compound in NASH.



1c. Treatment Effect on NAS

NAS Component and Score		NGM282 (n=6)	GFP (n=6)	NGM282 vs GFP
Steatosis	0	6	1	p=0.0117
	1	0	4	
	2	0	1	
	3	0	0	
Lobular Inflammation	0	3	0	p=0.0041
	1	2	0	
	2	1	4	
	3	0	2	
Hepatocyte Ballooning	0	1	0	p=0.0009
	1	5	0	
	2	0	6	
Total NAS (mean ± SD)		1.5 ± 1.0	5.33 ± 1.5	p=0.0005

Figure 1.

P0933
NGM282 EXHIBITS POTENT ANTI-INFLAMMATORY AND ANTI-FIBROTIC ACTIVITY IN FXR-NULL MICE WITH NON-ALCOHOLIC STEATOHEPATITIS (NASH) HISTOPATHOLOGY

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Background and Aims: NASH is an increasingly common cause of chronic liver disease worldwide and is associated with increased liver-related morbidity and mortality. There is an increased understanding of the role of bile acids (BA) and FGF19 in the pathogenesis of NASH and related fibrosis. NGM282 is a novel non-tumorigenic recombinant variant of FGF19 with potent inhibition of CYP7A1 and BA synthesis. We evaluated the effects of NGM282 in aged FXR-null mice that exhibit hepatic steatosis, degeneration and fibrosis consistent with NASH.
Methods: Sustained exposure of NGM282 was achieved by adeno-associated virus (AAV)-mediated gene delivery following a single tail vein injection into 6-month old FXR null mice. Hepatic expression of pro-inflammatory and pro-fibrotic genes was assessed by quantitative RT-PCR at 24 weeks after AAV administration in FXR null mice. Serum levels of ALT and alkaline phosphatase were determined as markers of hepatocellular and biliary damage. Liver sections were evaluated following H&E staining

Results: NGM282 significantly reduced hepatic expression of pro-inflammatory cytokines (*TNFa, IL-1b;* Figure 1A) and chemokines (*Ccl2, Cxcl2*) as well as key pro-fibrotic genes including *TGFb1* and *Galectin-3* (Figure 1B). mRNA levels of collagen (*Col1a1, Col1a2, Col3a1, Col14a1*, Figure 1C), matrix metallopeptidases (*Mmp2, Mmp12*) and tissue inhibitors of matrix metallopeptidases (*Timp1, Timp2*) were normalized by NGM282 treatment. Histological analysis demonstrated that NGM282 reduced hepatic steatosis, immune cell infiltration, and bile duct proliferation in FXR-null mice.
Conclusions: NGM282 protects aged FXR-null mice from developing steatosis, hepatic inflammation and fibrosis. These activities, in addition to the metabolic and BA synthetic actions, are supportive of the clinical development of NGM282 in NASH.

Figure 1.

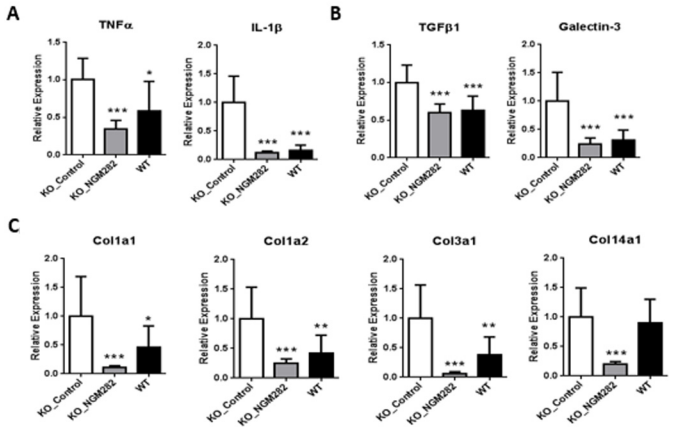

Figure 1.

P0934

LYSOPHOSPHATIDYLCHOLINE (LPC) AS CENTRAL PLAYER FOR HEPATIC FAT ACCUMULATION AND INFLAMMATION: IMPLICATION FOR PATHOGENESIS OF NASH

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Background and Aims: In NASH the intracellular ratio of phosphatidylcholine (PC): lysophosphatidylcholine (LPC) is decreased due to activation of the membrane localized phospholipase A2 (iPLA2β). Here we aim the evaluation of LPC levels in development and reversal of fat accumulation and inflammation.
Methods: In HepG2 cells the bile acid-phospholipid conjugate ursodeoxycholate-lysophosphatidylethanolamide (UDCA-LPE) as $iPLA_2\beta$ inhibitor was used to modify intracellular LPC levels. We examined the impact of LPC on JNK1-p and transcription of the heterotetrameric fatty acid transport complex constituted of CD36, $FABP_{PM}$, caveolin1 and $iPLA_2\beta$. A NASH phenotype was generated by exposure of HepG2 cells with high concentrations of oleate bound to albumin (4:1) for 3 h.
Results: Addition of 1–10 μM LPC to delipidated cytosolic extracts revealed a dose dependent increase of JNK1-p a central promoter of fatty acid metabolism and lipoapoptosis. In vitro transcription with native HepG2 nuclear extracts exposed to these LPC conditioned cytosolic samples resulted in synthesis stimulation of all members of the fatty acid uptake complex. In contrast, the NASH phenotype was reversed or prevented by incubation with the $iPLA_2\beta$ inhibitor UDCA-LPE (1 h, 100 μM) suppressing cytosolic LPC levels. Accordingly JNK1-p and transcription of fatty acid transporter biosynthesis were reduced, concomitantly with disappearance of lipid droplets, triglyceride accumulation and LDH release in the medium.
Conclusions: $iPLA_2\beta$ mediated generation of LPC represents a central regulator for hepatic steatosis and inflammation via JNK1-p. Inhibition of iPLA2β by nontoxic UDCA-LPE could be an ideal therapeutic strategy against NASH.

P0935

NON-ALCOHOLIC STEATOHEPATITIS (NASH): 2 COMPLEMENTARY DIETARY MOUSE MODELS

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Background and Aims: Non-alcoholic steatohepatitis (NASH), the potentially progressive form of nonalcoholic fatty liver disease (NAFLD), is the pandemic liver disease of our times. However, its ideal treatment has yet to be found. To better understand the physiology of NASH and develop therapeutic strategies, a good animal model is needed that is reproducible, easy to implement, and that causes not only hepatic steatosis, but also the characteristics of NASH (cellular injury/death, inflammation), as well as fibrosis (the pathology that confers bad prognosis in humans). We compared features of NASH in 2 mouse models most widely used to study the disease: methionine-choline deficient (MCD) diet and Western diet.
Methods: 36 C57Bl6 mice were fed either chow diet, MCD diet for 8 weeks or Western diet (45% energy from fat, predominantly saturated fat, with 0.2% cholesterol, plus drinking water supplemented with fructose and glucose) for 16 weeks. All animals were sacrificed at 20 weeks of age. Liver pathology and metabolic profile were compared.
Results: The metabolic profile associated with human NASH was best mimicked by Western diet, which induced weight gain, hyperleptinemia, insulin resistance and dyslipidemia. Hepatic steatosis (i.e., triglyceride accumulation) was also more severe in Western diet than MCD diet. However, liver non-esterified fatty acids content was higher in the MCD diet group. Hepatic steatosis associated with increased *de novo* lipogenesis in Western diet and decreased VLDL secretion in MCD diet. NASH was much more severe and reproducible in the MCD model, as evidenced by greater liver cell death/apoptosis and inflammation. Compared to mice fed Western diets, MCD diet-fed mice developed an enhanced ductular response, and significantly more fibrosis. Various mechanisms implicated in human NASH pathogenesis/progression were also significantly more robust in the MCD diet model, including oxidative stress, ER stress, autophagy deregulation, and hedgehog pathway activation.
Conclusions: MCD and Western diet are two complementary models to study NASH. Only Western diet reproduces the metabolic disturbances seen in human NASH. Western diet induces severe hepatic steatosis but after 4 months of diet exposure, liver injury is limited and minimal fibrosis is evident. MCD diet more efficiently reproduces the pathophysiologic mechanisms that drive human NASH pathogenesis/progression and causes substantial liver injury and fibrosis in 2 months.

P0936

THE IMPORTANCE OF THE INTERPLAY BETWEEN HEPATOCYTES AND HEPATIC STELLATE CELLS DURING FIBROGENESIS IN A NASH IN VITRO MODEL

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Background and Aims: Activation of hepatic stellate cells (HSC) and dysregulation of several mediators such as matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) play a determinant role in the fibrogenesis during the progression of NAFLD to NASH. This study was aimed to establish the interplay between hepatocytes and HSC in an *in vitro* cell model of NASH.
Methods: The effect of free fatty acids (FFA) (Oleic:Palmitic, 2:1) was analyzed at short (24 h) and long (96 h) exposure times in different experimental set-ups: (1) Monoculture of each cell type; (2) Transwell system (soluble mediators effects) and (3) simultaneous co-culture (SCC) by seeding both cell types together (cell-to-cell interaction). In each system was assessed the amount of steatosis; expression of HSC activation marker (α-SMA), ECM turnover regulators (MMP-2 and TIMP-2) as well as collagen biosynthesis in comparison to untreated cells (ctrl).
Results: The amount of steatosis was comparable among all the experimental set-ups. However, HSC activation in terms of α-SMA gene (2.20±0.25-folds; $p < 0.01$) and protein (1.70±0.20-folds; $p < 0.01$) expression was only increased in the SCC and was maximal after 24 h of FFA exposure. Similarly, the close contact of the two cell types induced an up-regulation of TIMP2 protein (1.42±0.27-folds; $p < 0.05$) which was inversely correlated both with MMP-2 protein (0.58±0.10-folds; $p < 0.01$) and activity (0.70±0.13-folds; $p < 0.05$). This dysregulation was accompanied by an increase of collagen biosynthesis at longer FFA exposure times (1.5±0.10-folds, $p < 0.01$). Any of these effects was directly induced by FFA (monoculture) nor by the soluble mediators (transwell).
Conclusions: Our data suggest that hepatocytes-to-HSC proximity/interaction is essential for fibrosis initiation in NASH.

P0937

LIVER STEATOSIS IS ASSOCIATED WITH INCREASED PORTAL PRESSURES AND IMPAIRED LIVER REGENERATION FOLLOWING PARTIAL HEPATECTOMY IN MICE

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Background and Aims: Liver steatosis negatively impacts outcome after major liver resection in humans. The aim of this study was to analyze liver injury, cellular stress and impact on liver regeneration in a murine model of liver steatosis compared to lean controls.
Methods: Male C57Bl/6 mice were fed with either normal chow (4.5% fat content) or high fat diet (HFD, 16.6% fat content) for 6 weeks to induce liver stetosis and then subjected to minor (30%) or standard (60%) partial hepatectomy (PH). Serum transaminases (ALT, AST) and tissue analysis (mRNA) were measured before surgery. Survival and liver regeneration was assessed 48 hours post surgery. Portal pressure was measured by direct cannulation of an ileocolic vein before and after liver resection, and liver regeneration was assessed by BrdU and Ki-67 immunohistochemistry.
Results: After six weeks of HFD, liver weight was significantly increased compared to control mice. By histology, significantly more fat content, cellular ballooning and inflammatory infiltrations were observed in steatotic, compared to lean mice. Serum transaminases were significantly increased in the HFD group. In liver tissue, mRNA of TNF alpha, GADD45 and p21 were significantly increased in steatotic livers compared to control livers. The 2 groups did not differ significantly in terms of baseline portal pressures and hepatic hydroxyproline content. Following both, minor and standard PH portal venous pressure was increased in steatotic compared to lean mice during the early phase of regeneration (minor PH 11 vs 10 mmHg, $p = 0.001$; standard PH 12 vs. 11 mmHg, $p < 0.0001$). Liver regeneration measured by BrdU and Ki-67 positivity was significantly reduced after minor PH in steatotic than in control mice [BrdU positive hepatocytes per high power field (HPF): 3.8 vs. 11, $p < 0.0001$; Ki-67 positive hepatocytes per HPF: 6.4 vs. 19.4, $p < 0.0001$]. Survival after standard PH was significantly reduced in steatotic than in lean mice, whereas there were no deaths after minor PH in both groups.
Conclusions: Liver steatosis is associated with an increased portal venous pressure after partial hepatectomy and reduced liver regeneration leading to decreased survival. Furthermore, liver steatosis has impact liver function and is associated with increased cellular stress and tissue inflammation.

P0938

OLIVE OIL COMSUMPTION AMELIORATES NON-ALCOHOLIC STEATOHEPATITIS INDUCED BY HYPERCALORIC AND HYPERLIPIDIC WESTERN DIETS

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Background and Aims: To evaluate the protective role of extra virgin olive oil consumption in NAFLD, analysing signalling and molecular mechanisms.
Methods: 5-week-old male C57BL6J (n = 150) were fed 9 months with: (i) standard diet [3.4% fats; 0.6% saturated fats (SFA); 0.7% MUFA] (Ct; n = 30); (ii) saturated fats HFD (43% fats; 18% SFA; 20% MUFA) (HFD-SFA; n = 30); (iii) extra virgin olive oil HFD (43% fats; 4% SFA; 34% MUFA) (HFD-EVOO; n = 30); (iv) HFD-EVOO rich in phenolic compounds (five times higher) (HFD-O; n = 30) and (v) 3 months with HFD-SAF, followed by another 6 months with standard diet (R; n = 30). Standard diet energy density was 2.9 kcal/g and HFD was 5.9 kcal/g. It was evaluated: (i) food and fluid intake; (ii) body weight gain; (iii) glycaemia, insulinaemia, HOMA-IR and lipid profile; (iv) inflammatory plasmatic markers (IL-6, TNF-α, IFN-γ); (v) liver weight; (vi) total lipids liver content; (vii) liver fatty acids and triglycerides profile; (viii) histology (hematoxylin–eosin, Sirius Red and Oil-Red) and (ix) liver proteomic analysis. Statistical analysis was performed using SPSS v22, by ANOVA and Tukey–Kramer.
Results: Comparing HFD-EVOO and HFD-O with HFD-SFA mice, it was observed: (i) Significant reduction of body weight, glycaemia and HOMA index; (ii) Significant reduction of total cholesterol, LDL and triglycerides plasmatic values; (iii) Significant increase of plasmatic HDL concentrations; (iv) Significant reduction of IFN-γ plasmatic levels; (v) Significant reduction of total hepatic lipids; (vi) Significant increase of MUFAs and trioleins hepatic content; (vii) Significant reduction of NAS score and Sirius Red positive cells and (viii) Significant reduction of proteins involved in insulin resistance, hepatic steatosis, inflammation and NAFLD.
Comparing HFD-EVOO with HFD-O mice, it was observed: (i) Significant reduction of LDL and IFN-γ plasmatic levels and significant increase of HDL; (ii) Significant reduction of liver lipids content and (iii) Significant reduction of NAS score and Sirius Red positive cells. All parameters analysed returned to normality in R group.

Table (abstract P0938).

	Control	HFD-SAF	HFD-EVOO	HFD-O	R	Statistics
Weight (g)	42±1.3	52±1.6	47±1.4	44±2.6	43±1.8	N = 30/group; *$p < 0.05$; NS
Glycaemia (mg/dl)	107±3	192±8	158±6	155±4	115±4	N = 30/group; *$p < 0.01$; NS
HOMA-IR	1.7±0.3	31.7±7.4	14.2±4.2	11.2±1.2	5.1±1.2	N = 30/group; *$p < 0.01$; NS
Plasmatic IL-6 (pg/ml)	8.9±1.8	22.7±2.6	20.9±4.3	18.7±2.8	11.4±2.9	N = 10/group; NS; NS
Plasmatic TNF-α (pg/ml)	26.3±3.7	32.4±4.8	29.1±4.0	29.5±1.7	27.0±3.2	N = 10/group; NS; NS
Plasmatic IFN-γ (pg/ml)	6.1±1.8	35.4±7.6	11.5±4.6	4.8±1.9	5.6±1.2	N = 10/group; *$p < 0.01$; *$p < 0.01$
Liver weight (g)	1.3±0.2	2.1±0.6	2.3±0.7	1.8±0.4	1.2±0.15	N = 5/group; NS; NS
Total lipid content (%)	10±1	29±4	21±2	16±3	18±5	N = 4/group; *$p < 0.05$; *$p < 0.01$
MUFA hepatic content (g/100 g liver tissue)	38±2	53±2	66±2	61±1	ND	N = 5/group; *$p < 0.05$; NS
Hepatic trioleins (g/100 g liver tissue)	3.2±0.5	6.7±0.2	12.5±0.2	12.8±1	ND	N = 5/group; *$p < 0.01$; NS
NAS score	0±0	4.6±0.3	3.6±0.5	2±0	0±0	N = 3/group; *$p < 0.05$; *$p < 0.05$
Positive Sirius Red cells (%)	0.2±0.03	2.6±0.5	1.2±0.6	0.4±0.02	0.3±0.02	N = 3/group; *$p < 0.05$; *$p < 0.01$

First statistics are referred to HFD-SAF vs HFD-EVOO and HFD-O; second statistics corresponds to HFD-EVOO vs HFD-O.

Conclusions: Enriched olive oil with HFD decreased NAFLD-induced liver manifestations. Compounds with antioxidant activity partially explains the improvement of some parameters. Insulin resistance decrease and changes in liver lipids content profile, together with the modification of the expression of proteins related with hepatic steatosis and inflammation have a relevant impact in the improvement observed in NAFLD.

P0939
FATTY LIVER VS. FIBROTIC LIVER: COMPARISON OF INFECTION-ASSOCIATED PORTAL HYPERTENSION

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Background and Aims: Portal hypertension and alterations of liver structure are of major relevance for chronic liver diseases. We have identified TXA_2 as important vasoconstrictor upon infection following TLR 2/6 activation in the liver (e.g. Steib CJ et al., Hepatology 2010). The aim of this study was therefore to investigate, whether the cause of liver disease influences the extent of TXA_2 induced portal hypertension.
Methods: Bile duct ligation (BDL) in male Sprague-Dawley rats caused fibrosis after 4 weeks (BDL4) and fatty liver was induced by high fat diet. Isolated liver perfusion was performed for 80min. In all three liver models three groups (each n = 5) were investigated: **A**: control perfusion; **B**: + Zymosan A (150 μg/ml, 40.–46.min.); **C**: + U46619 (TXA_2 analogue, 0.1μM/ml, 40.–46.min.). Portal perfusion pressure (p) and Thromboxane B_2 (TXB_2, stable degradation product) in the perfusate (+ Zym) were measured (mean ± SEM; *$p < 0.05$). Immunhistochemical evaluation of the livers using CD163 (to determine Kupffer cell density) and α-SMA (to estimate number of activated hepatic stellate cells) is in process.
Results: HE staining showed, in comparison to healthy livers, a mild inflammation and moderate to severe fat deposition in fatty livers and a moderate to severe fibrosis with moderate inflammation in BDL4. Portal perfusion pressure and TXB_2 production after Zymosan A (group B) and portal perfusion pressure after TXA_2 analogue U46619 (group C) are shown in Table 1. Interestingly, p_{max} was significantly lower after Zymosan A in fatty livers compared to healthy livers and following U46619 p_{max} was lower in fatty livers compared to fibrotic livers.
Conclusions: Fatty livers were less sensitive to TLR 2/6 activation and TXA_2 analogue administration than healthy and fibrotic livers. The correlation between portal perfusion pressure and TXB_2 decreases from healthy to fatty on fibrotic liver, which might be depend on the increasing damage and alteration of liver architecture because of the liver disease. In summary, this leads to the assumption that fatty livers have a protective effect on infection-associated portal hypertension. Hereby, it offers future clinical situations the possibility to stratify the risk for patients with fatty livers and prompts further research.
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Table 1. Portal perfusion pressure (p) and TXB_2 production after Zymosan (group B) and portal perfusion pressure after TXA_2 analogue U46619 (group C)

	Group B			Group C
	p_{min} vs. p_{max} (cmH_2O)	TXB_2-min vs. TXB_2-max (pg/mL × g liver)	Correlation coefficient between p and TXB_2	p_{min} vs. p_{max} (cmH_2O)
Healthy	4.4±0.4 vs. 17.6±0.9*	41.8±5.8 vs. 1003.1±172*	1±0	3.4±0.1 vs. 20.6±0.9*
Fatty liver	4.1±0.2 vs. 11.4±0.6*	29.4±2 vs. 532.7±16.2*	0.7±0	3.3±0.1 vs. 24±0.9*
Fibrosis	8.6±0.3 vs. 15.4±0.6*	87.8±12.7 vs. 1049.2±286.7*	0.5±0.1	8.3±0.2 vs. 30.5±1.9*

Mean±SEM; *$p < 0.05$.

P0940
CHRONIC PARTICULATE MATTER EXPOSITION REPRESENTS A SECOND HIT IN THE PROGRESSION FROM STEATOSIS TO STEATOHEPATITIS

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD), a hepatic manifestation of metabolic syndrome, is the most common chronic liver disease, and the prevalence is rapidly increasing in developed countries. Nonalcoholic steatohepatitis (NASH), the severe form of NAFLD, can also progress to liver cirrhosis and hepatocellular carcinoma. Recent evidences suggest that environmental factors can trigger hepatic inflammation and progression of steatosis to NASH. We evaluate if a western style diet in association with chronic urban particulate matter exposition can modifies the pathogenesis and progression of NASH.
Methods: The experimental model was created to reproduce urban lifestyle: C57Bl/6 mice were fed with normal chow (ND) or a western style diet (HFD) rich in fat, cholesterol and sugar, and treated with or without particular matter (PM) collected from the urban area of Florence (Italy). After 4 and 8 weeks were performed the morphologic analysis of liver tissues, the evaluation of inflammatory cell infiltrate and the collagen deposition; we evaluate also the effects of PM on cytokine production and oxidative stress related cellular damage.

Results: Already after 4 week of treatment were present differences among the HFD group and the HFD-PM group; as expected the HFD groups developed fat accumulation in the liver but, interestingly at 8 weeks, the NASH score was significantly increased in the second group ($p < 0.01$) based on the histological analysis of the liver, the tissue fat content, the inflammatory cell infiltrate and the collagen deposition. The cytokine profiles indicate an increased production of pro-inflammatory molecules in presence of PM. The levels of DNA adducts were significantly increased in the HFD groups compared to standard chow. Many adducts were identified in liver tissues: one of them was benzo(a)pyrene, that is a well known polycyclic aromatic hydrocarbon with strong mutagenic properties. Interestingly the effects of HFD and PM were synergic in the sense that the levels of DNA adducts were about thirty fold greater in HFD mice in respect to controls treated with the same amounts of air particulate matter extracts mainly due to benzo(a)pyrene increment.
Conclusions: Our data suggest that in subject with steatosis due to a western style of life, the association with a chronic exposition to urban particulate matter plays an important role in the pathogenesis of NASH and its evolution to cirrhosis and also to cancer.

P0941
CD44 IS AN IMPORTANT ACTOR IN NON ALCOHOLIC STEATOHEPATITIS

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Background and Aims: CD44 is expressed on many cell types that contribute to inflammation. CD44 plays an important role in the recruitment of macrophages into adipose tissue with obesity and of leukocyte into liver in response to lithogenic diet. However, its role in hepatic inflammation in obese patients and in mouse models of steatohepatitis has not yet been investigated.
Methods: CD44 rs187116 and rs13347 genotypes were determined by allelic discrimination using TaqMan reagents in a cohort of 274 morbidly obese patients with liver biopsy-proven NAFLD. Gene expression of CD44, IL1β, TNFα was evaluated in 30 obese patients (8 without NAFLD, 13 with hepatic steatosis and 9 with NASH). The role of CD44 was evaluated in *cd44*$^{-/-}$ mice with steatohepatitis induced by methionine- and choline-deficient diet (MCD) (Wt Ctr Diet=8, MCD=14; *cd44*$^{-/-}$ Ctr Diet=12, MCD=19) by histological, biological, gene expression and flow cytometry analysis.
Results: Carriage of CD44 rs187116 minor A allele was associated with presence of hepatic inflammation independently of age, gender, body mass index and the presence of type 2 diabetes in morbidly obese patients ($p = 0.0312$). Further, CD44 hepatic gene expression was strongly upregulated in NASH patients and correlated with NASH grading ($r_s=0.668$, $p < 0.001$), NAS ($r_s=0.562$, $p = 0.001$), ballooning ($r_s=0.531$, $p = 0.003$) and ALT ($r_s=0.496$, $p = 0.005$). Experiments in mouse model of steatohepatitis (MCD) further showed the hepatic upregulation of CD44 at the mRNA and protein level. AST and ALT levels were strongly decreased in *cd44*$^{-/-}$ mice compared with wild-type mice after MCD challenge (−28% and −39%, respectively). Histological analysis of the liver of *cd44*$^{-/-}$ mice revealed a significantly lower incidence of hepatic steatosis (10±4% vs 48±8%, $p < 0.001$) and inflammation (2±1 vs 7±2 inflammatory foci/10 fields, $p < 0.001$). In addition, the hepatic expression level of markers of inflammation (MCP1, TNFα and IL1β) and macrophages (F4/80) was strongly decreased in *cd44*$^{-/-}$ mice versus wild-type mice after MCD. Flow cytometry analysis also revealed a prevention of F4/80 macrophage infiltration into the liver of *cd44*$^{-/-}$ mice in response to MCD.
Conclusions: Human and experimental data suggest an important role for CD44 in the pathogenesis of NAFLD (steatosis and NASH).

P0942
IMMUNE REACTIONS IN STEATOHEPATITIS INITIATION AND PROGRESSION TO FIBROSIS: CRITICAL ROLE OF TH17 AND TH22 LYMPHOCYTES

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Background and Aims: The mechanisms responsible for steatosis progression to NASH and for NASH evolution to fibrosis are incompletely understood. Recent evidences, indicate that hepatic immune response may also play critical role in NASH. In particular, IL-17 and IL-22 produced by CD4+ T cells can modulate the inflammatory reactions and survival processes. This study investigated Th17 ($CD4^+IL\text{-}17^+$) and Th22 ($CD4^+IL\text{-}22^+$) lymphocytes infiltration in mice liver during NASH development and explored the role of IL-17/ IL-22 production in this process.
Methods: C57BL/6 WT and IL-17KO mice were fed either a normal diet (ND) or a methionine/choline deficient diet (MCD) diet. Liver T lymphocytes were analyzed by flow cytometry. Primary mouse hepatocyte were exposed to palmitic acid (PA)
Results: MCD diet induced progressive hepatic damage as evidenced by ALT increase, progressive micro and macro steatosis, necrosis, lobular inflammation and finally fibrosis. In the liver, Th17 cells significantly increased already at 1 week of MCD diet compared to ND mice, decreased at 2 and 4 weeks, and further increased at 8 weeks. By contrast, Th22 displayed an opposite kinetic.
We investigated the role of IL-17 and IL-22 in steatotic liver damage and protection, by employing primary mice hepatocyte exposed to PA. PA alone induced hepatocytes damage and increased JNK phosphorylation, a known marker of hepatocyte lipotoxicity. IL17 treatment further increased these parameters, whereas IL-22 protected them and activated a central mediator of cellular survival processes: Akt. Consistently inhibition of Akt, reversed IL-22-induced prevention of JNK phosphorylation and abolished cytoprotection.
To clarify the importance of IL-17 in initiate and sustain liver damage, NASH was induced in IL-17 KO mice. Lower ALT levels and lower NASH and fibrosis markers and an higher percentage of Th22 cells in the liver were observed in IL-17KO mice compared to WT mice. The reduced liver injury and fibrosis in IL-17 KO mice was related to an increased Akt activation and decreased JNK phosphorylation, according to *in vitro* results.
Conclusions: a biphasic increase of Th17 over Th22 is associated to NASH initiation and evolution to fibrosis in mice. Such effects are mediated by a prevalence of the lipotoxic JNK-dependent activity of IL-17 on the Akt-related cytoprotective action of IL-22.

P0943

BENEFICIAL EFFECTS OF IL-1 CYTOKINE INACTIVATION AND THE ROLE OF THE SERINE/THREONINE KINASE MK-2 IN HEPATIC STEATOSIS IN A MURINE OBESITY MODEL

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Background and Aims: Although non-alcoholic fatty liver disease (NAFLD) is among the most common causes of chronic liver disease worldwide, its pathogenesis is yet poorly understood. Recent studies show that IL-1α/β play a crucial role in disease development. Their recognition by cells does not only result in transcriptional regulation of many IL-1 target genes, but also negatively affects cell surface expression of the IL-6 signal transducer in an MK-2 (mitogen-activated protein kinase-activated protein kinase 2)-dependent mechanism. Therefore the cells might become less susceptible for IL-6 signalling, which is suspected to be beneficial in steatosis. We aim to investigate the role of IL-1 in early steatosis in IL-1-immunized and MK-2 deficient mice.
Methods: C57BL/6J wild-type and MK-2 deficient mice were fed a Surwit High Fat Diet (HFD) for 8 weeks; for inactivation of IL-1 mice were subcutaneously vaccinated against IL-1α/β using virus-like particles presenting antigens of IL-1α/β (Cytos-Biotechnology). Liver and fat tissue was analysed and RNA was isolated to assess gene expression via qPCR.
Results: Our recent findings show that inactivation of IL-1 by virus-like particles drastically reduces steatosis in mice fed a HFD for 6 weeks on a macroscopic and molecular level. Lipid accumulation is drastically reduced and lipogenic enzyme as well as proinflammatory molecule expression is significantly downregulated. Although no cytokine expression is detectable in the liver at such an early timepoint expression levels are elevated in adipose tissue of animals fed a HDF. IL-1β serum levels show no differences between chow- or HFD-fed mice, pointing towards an indirect effect of fatty tissue inflammation on the liver. MK-2 deficient mice show no significant differences in liver weight or expression of any inflammatory markers in qPCR after 8 weeks of feeding.
Conclusions: Our results strongly support future studies on IL-1 for clinical applications since its inactivation prevents liver steatosis in HFD-fed mice. The present study indicates that the main initial target site for IL-1 is the adipose tissue rather than the liver itself. Therefore, the effect on the liver appears to be indirect as IL-1β serum levels do not increase upon feeding mice a HFD. Furthermore, MK-2 deficient mice fed a HFD for 8 weeks show no difference in liver inflammation in comparison to WT mice, indicating that the IL-1/IL-6 cross-talk does not affect the early phase of steatosis.

P0944

MODULATION OF MUSCLE MICRORNA EXPRESSION PROFILES IN PATIENTS WITH NAFLD AND IN C2C12 CELLS INCUBATED WITH PALMITIC ACID

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Background and Aims: In the skeletal muscle, non-alcoholic fatty liver disease (NAFLD) associates with intramyocellular lipid deposition, mitochondrial dysfunction and insulin resistance (IR), particularly in obese patients. Further, recent evidences support a functional role for microRNAs (miRNA/miRs) in regulating muscle mitochondrial impairment and IR. Finally, tauroursodeoxycholic acid (TUDCA) is cytoprotective in both liver and muscle cells, in part, by stabilizing mitochondria. Our aims were to profile global muscle miRNA expression profiles from patients at different NAFLD stages and validate their role, as well as of TUDCA, in insulin-resistant muscle cells.
Methods: Muscle and matching liver biopsies were obtained from morbid obese NAFLD patients undergoing bariatric surgery. Muscle RNA was run in TaqMan MicroRNA arrays. qPCR array data was analyzed using the HTqPCR package in Bioconductor. Differential expression analysis was performed with interquantile range values >1.5 using the lmfit function of the Limma package and the Benjamini-Hochberg conditional hypergeometric test algorithm. C2C12 cells were incubated with or without palmitic acid (PA), in the presence or absence of TUDCA, for characterization of the insulin signaling pathway, as well as mitochondrial and overall cellular toxicity.
Results: Our results show a progressive and significant increase in the expression of 6 muscle miRNAs from steatosis to more severe NASH (at least $p < 0.05$). These included miR-339-3p, which has been described to regulate glucose synthesis, and miR-361, found increased in type II diabetes (T2D) patients serum. Inversely, 8 miRNAs were decreased (at least $p < 0.05$), including miR-20b, reported as down-regulated in T2D patients plasma. Incubation of C2C12 cells with PA inhibited the insulin-signaling pathway, while increasing mitochondrial dysfunction and apoptosis (at least $p < 0.05$), all of which were prevented by co-incubation of cells with TUDCA ($p < 0.05$). Of note, miR-339-3p was also increased by PA ($p < 0.05$), targeting MAPK phosphatase-7 (MKP-7), a negative regulator of JNK, thus likely contributing for IR.
Conclusions: In conclusion, our results indicate that miRNAs associated with T2D and mitochondrial dysfunction are differently modulated with NAFLD severity in the muscle, with miR-339-3p arising as a novel mechanistic player. In addition, TUDCA attenuates muscle cells IR and lipoapoptosis and, as such, may ameliorate NAFLD-associated muscle dysfunction.
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P0945

SURGICAL REMOVAL OF INFLAMED EPIDIDYMAL WHITE ADIPOSE TISSUE OF OBESE C57BL6/J MICE ATTENUATES THE DEVELOPMENT OF NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) and the development of its severe form non-alcoholic steatohepatitis (NASH) is strongly associated with abdominal obesity. It has been postulated that obesity-associated inflammation in abdominal white adipose tissue (WAT) contributes to this relationship. To investigate whether inflamed WAT plays a causal role in the pathogenesis of NAFLD, we surgically removed the inflamed abdominal (epididymal) WAT of obese mice (with established steatosis) and investigated further progression of NAFLD towards NASH.
Methods: Male C57BL/6J mice were fed a high-fat diet (HFD) for 12 weeks to develop obesity with inflammation in epididymal WAT (eWAT) and liver steatosis. Subsequently, surgical removal of inflamed eWAT (WATx) or a SHAM procedure was performed and mice continued on HFD for another 12 weeks. NAFLD development was analyzed histologically and by RT-PCR.

Results: Body weight and total fat mass were comparable between the groups at the end of the experiment, but eWAT mass remained low after surgery in WATx. Liver histology scores demonstrate that WATx mice showed a similar degree of steatosis compared to SHAM, but NASH development was significantly reduced as revealed by a reduction in inflammatory aggregates (−40%) and inflammatory factors (TNF-alpha: −37% and MCP-1: −31%). Attenuation of NASH development was accompanied by reduced circulating levels of inflammatory mediators, such as leptin and free fatty acids.
Conclusions: Surgical removal of inflamed eWAT attenuated the progression of NAFLD towards NASH, indicating a causal role of eWAT in NAFLD development. The crosstalk between WAT and liver may be mediated by WAT-derived pro-inflammatory factors such as leptin and free fatty acids.

P0946
CX3CR1-EXPRESSING INFLAMMATORY DENDRITIC CELLS CONTRIBUTE TO THE PROGRESSION OF NONALCOHOLIC STEATOHEPATITIS (NASH) IN MICE

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Background and Aims: Liver dendritic cells (DCs) are a heterogeneous population of specialized bone marrow-derived cells mainly involved in antigen presentation to lymphocytes. In healthy livers DCs have a predominant tolerogenic phenotype, but recent evidence suggests their possible contribution in the evolution of chronic liver diseases. In this study, we have investigated the role of monocyte-derived inflammatory dendritic cells (moDCs) in experimental NASH.
Methods: NASH was induced in C57BL/6 mice by feeding a methionine-choline deficient (MCD) diet for up to 8 weeks.
Results: In MCD-fed mice the progression of NASH to fibrosis was associated with a ten fold expansion of myeloid-derived F4–80$^+$ cells featuring the combination of inflammatory monocyte markers (CD11b, Ly6C) and the fractalkine receptor (CX_3CR1). These CX_3CR1^+ cells were also characterized by the expression of dendritic cell markers (CD11c, MHCII) and by a sustained TNF-α production, suggesting monocyte differentiation to inflammatory moDCs. The accumulation of CX_3CR1^+ moDC in advanced NASH paralleled with the lowering in phasmocytoid and lymphocytoid DC and a decline in macrophage M1 activation markers. Conversely, the expansion of TNF-α-producing CX_3CR1^+ moDCs was associated with an elevation in hepatic and circulating TNF-α levels and the worsening of parenchymal injury. Hydrogen sulfide (H_2S) has been shown to interfere with CX_3CR1 up-regulation in monocyte-derived cells exposed to pro-inflammatory stimuli. Treating 4 weeks MCD-fed mice with the H_2S donor NaHS while continuing on the same diet prevented the accumulation of TNF-α-producing CX_3CR1^+-moDCs without interfering with hepatic macrophage functions. Furthermore, NaHS reduced hepatic and circulating TNF-α levels and ameliorated transaminase release and parenchymal injury.
Conclusions: Altogether, these results indicate that inflammatory CX_3CR1^+-moDCs contribute in sustaining lobular inflammation and liver injury during NASH progression.
This work has been supported by a grant from the Fondazione Cariplo, Milan Italy.

P0947
A COMPUTATIONAL MODEL OF ZONATION IN HEPATIC METABOLISM: UNDERSTANDING ZONATED STEATOSIS IN NAFLD AND IDENTIFICATION OF POTENTIAL DRUG TARGETS

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is strongly associated with insulin resistance (IR). In adult NAFLD, steatosis (STT) originates in perivenous (PV) cells before spreading across the sinusoid. Since PV cells are lipogenic, whilst periportal (PP) cells preferentially uptake fatty acids (FA), this pattern of lipid build-up is often considered counter-intuitive given the context of reduced insulin stimulation of lipogenesis but raised plasma lipid levels. We aim to develop an *in silico* model to understand the causes of PV-STT and the implications of hepatic heterogeneity on drug development.
Methods: Fully characterizing the metabolic changes occurring in each region of the sinusoid in NAFLD experimentally would be complex, time-consuming and animal intensive. To reduce *in vivo* experimentation, a computational model of the sinusoid was rigorously built based on compartments of hepatocytes interacting with blood as it passes from PP to PV ends of the sinusoid.

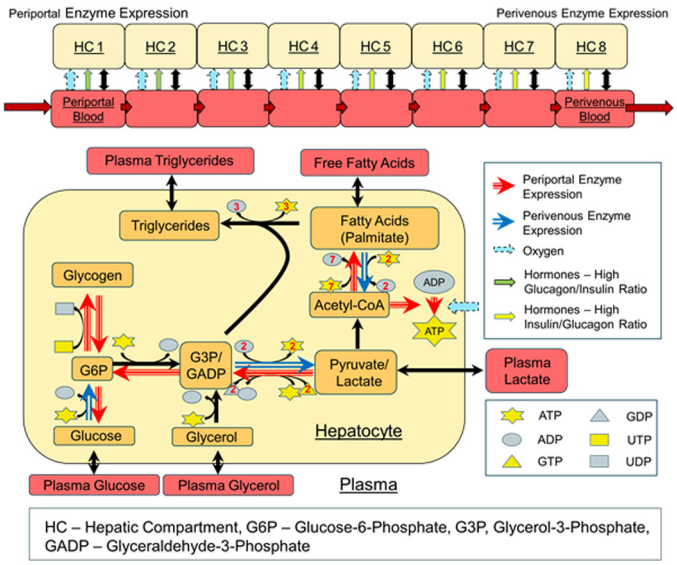

Figure: A simplified schematic of metabolism across the sinusoid as represented in the model.

Results: PV-STT was predicted to occur when the short term effects of IR were simulated alone. This was due to the build-up of carbohydrate metabolic intermediates in PV cells causing the rapid storage of FAs as triglycerides (TGs). PV-STT was also seen when simulating a high fat diet in metabolically normal individuals as a result of increased lipogenesis and lipid uptake.
The effects of targeting each pathway in the model in turn were analysed to assess potential drug targets for reducing STT. Only suppressing FA synthesis from acetyl-CoA was predicted to reduce hepatic and plasma lipid concentrations without disturbing energy metabolism. Suppression of lipid uptake or stimulation of TG release as VLDL were predicted clear hepatic STT, but also raised plasma lipid levels redirecting fats to other tissues. Blocking TG synthesis led to a vast increase in the FA concentration of PV cells likely to expedite cell damage. All relevant alterations to carbohydrate metabolism were predicted to disturb ATP synthesis, particularly in PV cells.

Conclusions: Firstly, we hypothesize that the build-up of carbohydrate metabolic intermediates as a result of defective glycogen storage, particularly in PV cells, contributes to the development of PV-STT in IR. Secondly, we propose that blocking the synthesis of FAs from acetyl-CoA is a promising target pathway to reduce STT without disturbing ATP production. Interventions in other metabolic processes are predicted to redirect lipids to other tissues or cause complications in energy metabolism.

P0948

UPREGULATED EXPRESSION OF SENP3 PLAYS AN IMPORTANT ROLE IN THE PROGRESSION OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD), ranging from nonalcoholic fatty Liver (NAFL) to progressive non-alcoholic steatohepatitis (NASH) and fibrosis, has become an increasingly common healthy problem. SENP3, a sentrin/de-SUMO specific protease, plays an important role in spectrum of cellular process. Reports have demonstrated that SENP3 was sensitive to a variety of stresses. However stresses such as oxidative stress, lipid accumulation, cytokines, et al, have been known as major factors involved in the development of NAFLD. Thus, the aim of this study was to investigate if and how SENP3 was involved in NAFLD.
Methods: Liver biopsy samples of 20 NAFLD patients and 3 normal controls, obtained from Shanghai Ruijin Hospital from 2012 to 2014, were subject to immunohistochemical staining (IHC) of SENP3 expression. Meanwhile, Sprague Dawley (SD) rats fed with high-fat diet were utilized as NAFLD animal model to further validate the results found in human. Finally, we treated normal liver cell line (L02) with medium containing oleate and palmitate (oleate/palmitate, 2:1 ratio) as the cell model of NAFLD to explore the potential mechanistic link between SENP3 and NAFLD. SENP3 was ectopically overexpressed or silenced in L02, and determined the cellular lipid content by Oil Red O staining.

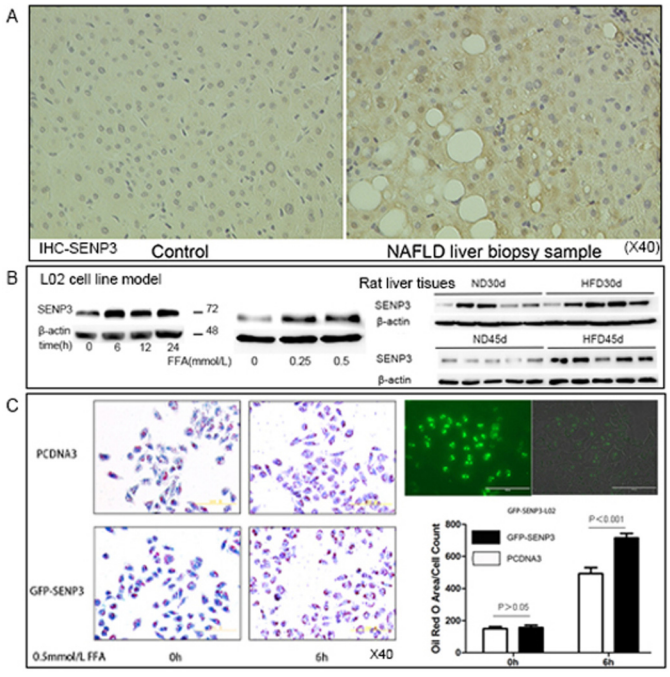

Figure: (A) Typical pictures of IHC with SENP3 antibody in human liver biopsy samples. (B) Western Blot showing SENP3 expression in L02 cells and rats. (C) Overexpression of SENP3 promoting lipid accumulation.

Results: IHC results showed that intrahepatic levels of SENP3 were higher in NAFLD patients than normal controls, and interestingly, SENP3 was found located around fat vacuole (typical pictures shown in Figure A). Moreover, elevated expression of SENP3 was also observed in the NAFLD rat model and cell model by Western Blot, PT-PCR and IHC, which is in consistency with the IHC results in human liver tissues (results of Western Blot shown in Figure B). Furthermore, SENP3 overexpression by transfected with GFP-SENP3 promoted lipid accumulation, while the ablation of it by transfected with SENP3 si-RNA inhibited lipid accumulation (results of SENP3 overexpression shown in Figure C).
Conclusions: Taken these findings together, we report for the first time that upregulated expression of SENP3 in the liver tissue may play an important role in the progression of NAFLD, especially in regulating hepatic lipid accumulation. The potential underline mechanism of SENP3 in NAFLD is still under investigation.

P0949

PIOGLITAZONE HYDROCHLORIDE PREVENTS NK CELL INSULIN RESISTANCE AND ENHANCES THEIR ACTIVITY IN NONALCOHOLIC FATTY LIVER DISEASE PATIENTS VIA mTOR PATHWAY

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Background and Aims: Obesity and insulin resistance are considered to play a role in Nonalcoholic Fatty Liver Disease (NAFLD) progression. Impairment of NK cells was explored as a mechanism for fibrosis progression into cirrhosis, suggesting that attenuating NK function is a prerequisite for the progression of the disease. We investigated a potential role of pioglitazone hydrochloride (insulin sensitizer) in NK cell modulation of "Insulin Resistance" in NAFLD patients.
Methods: Fifteen histology documented NAFLD patients lacking metabolic syndrome with advanced fibrosis score (F3–4) analyzed and correlated to fresh peripheral blood NK characterizations by flow-cytometry. Healthy volunteers used as a control. NK cells were investigated for insulin receptors and activity by CD107a. NK cells were in vitro pre-incubated with 10 mg/ml pioglitazone hydrochloride prior to modulations with physiological concentrations of insulin (20 IU) and 10 mg/ml rapamycin (mTOR inhibitor).
Results: Insulin receptors were expressed on 68.6±8% of healthy NK population, but significantly reduced to 48.4±3.3% in NAFLD NK cells ($P < 0.05$). This was correlated with increased insulin resistance (HOMA-score) suggesting NK cell insulin resistance. CD107a expressions although were unchanged (19.6±2.1%) within advanced fibrosis of NAFLD patients as compared to healthy donors, their NK cells had reduced secretions of γINF suggesting NK impairment. These changes in NAFLD NK cells were accompanied with a significant lower mTOR activity ($p = 0.001$). Using *in vitro* cultures, NAFLD NK cells had higher apoptosis rate and fail to reduce hepatic stellate cells (HSCs) activations as compared to normal donors. Pioglitazone hydrochloride inhibited decrease in NK insulin receptor on NAFLD NK cells an effects that were accompanied by elevations of NK CD107 and mTOR activity ($P < 0.04$). Rapamycin additions to the cultures reversed these effects while prevented by exposure of NK cells to insulin concentrations. NK exposure with physiologic insulin levels enhanced the killing of HSCs, while NK insulin resistance and impairment (induced by rapamycin) blocked the insulin effects to kill HSCs.
Conclusions: Pioglitazone hydrochloride enhanced NK functionality through modulations of mTOR activity and increase expressions of insulin receptor and consequently responded to physiological levels of insulin. Our data suggest a protective role of pioglitazone hydrochloride to increase potentials of NK cells to kill HSCs and limit NAFLD progression.

P0950

IMPACT OF KUPFFER CELLS ON HIGH FAT INDUCED INSULIN RESISTANCE AND LIVER FETUIN-A EXPRESSION

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Background and Aims: Hepatokines (liver secreted proteins with possible distant action) are emerging potential players in insulin resistance in type 2 diabetic patients. Here, we explore the effect of a high fat diet on the expression of fetuin-A, one of those candidate liver proteins, and its relation with liver macrophage (Kupffer cell) activation.
Methods: Male mice of 5 weeks of age were fed a normal diet (ND) or a high fat diet (HFD) for 3 days, known to initiate steatosis and insulin resistance. A preventive Kupffer cell (KC) depletion was obtained by intravenous injection of clodronate loaded liposomes and compared with PBS liposomes. The mRNA and protein expression of fetuin-A was evaluated by RT-PCR, Western-blot and immunofluorescence (IF) on different insulin-sensitive tissues (liver, adipose tissue and muscle).
Results: Short term HFD induced steatosis, KC activation and insulin resistance together with a significant increased expression of liver fetuin-A mRNA (1.5 fold, $p < 0.01$). However, liver fetuin-A protein expression remained unchanged under short term HFD. This increase in fetuin-A under high fat diet was not evidenced in the peripheral insulin sensitive tissues (skeletal muscle and adipose tissue) whether at the mRNA or at the protein level. Kupffer cell depletion in this setting did not reduce hepatic steatosis but significantly ameliorated insulin sensitivity proved by clamp studies. This amelioration in insulin sensitivity in KC-depleted mice was associated with a significant decrease in fetuin-A mRNA expression (0.7 fold, $p < 0.01$) compared to animals with KC. On immunofluorescence, fetuin-A was mostly expressed in centrilobular hepatocytes. Interestingly, while selectively depleting liver macrophages without affecting adipose tissue macrophage infiltration, intravenous clodronate injection was associated with a significant reduction in epididymal adipose tissue expansion compared to PBS injection (1.1% of body weight versus 1.6% of body weight, $p < 0.001$).
Conclusions: This study demonstrates liver fetuin-A overexpression at the initiation of HFD feeding, concurrent with hepatic steatosis and insulin resistance. Targeting KC in this setting improved insulin sensitivity and was associated with a decreased adiposity and a reduced liver fetuin-A expression suggesting that fetuin-A acts as an hepatokine with pro-adiposity and pro-insulin resistance effects.

P0951

MIR-21 INHIBITION AND FXR ACTIVATION SYNERGISTICALLY AMELIORATE DISEASE PATHOGENESIS IN A MOUSE MODEL OF NAFLD

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of liver lesions, from simple steatosis to non-alcoholic steatohepatitis (NASH). Intrahepatic accumulation of fat represents a major triggering factor; still, disease pathogenesis remains incomplete. Recently, microRNAs (miRNA/miRs) have been linked to NAFLD. In particular, mir-21 may contribute to disease progression by targeting peroxisome proliferator-activated receptor α (PPARα). Indeed, PPARα and farnesoid X receptor (FXR) constitute promising NAFLD therapeutic targets. We aimed to elucidate the role of miR-21 during NAFLD pathogenesis in mice, further evaluating the synergistic effect of miR-21 inhibition and FXR activation, using obeticholic acid (OCA).
Methods: Wild type (WT; n = 24) and miR-21 knockout (KO; n = 24) mice were fed either a standard diet (SD; n = 12) or a fast food diet (FF; n = 12) for 25 weeks. 6 animals from each group had their diets supplemented with 60 mg/kg OCA (kindly provided by Intercept). Mice were weighed weekly while blood was collected and liver extracted and weighed at sacrifice. In parallel, human liver biopsies were obtained from morbid obese NAFLD patients at different disease stages (n = 28). Liver samples were processed for histological analysis and determination of miR-21 and PPARα expressions by qRT-PCR and immunoblotting, respectively. Serum was used for biochemical parameters analysis.
Results: Our results show that, after 25 weeks, WT FF-fed mice develop NASH in parallel with an increase in both body weight and liver/body weight ratio, comparing with WT SD-fed mice ($p < 0.05$). Further, they exhibited increased miR-21 ($p < 0.05$) and decreased PPARα expressions ($p < 0.05$). Strikingly, miR-21 and PPARα also displayed an inverse and significant correlation in human patients, increasing from steatosis to less and more-severe NASH ($p < 0.05$). WT FF+OCA-fed animals displayed lower levels of miR-21, compared with WT FF-fed mice. KO FF-fed mice body weights and liver/body weight ratios were below WT FF-fed mice, as were serum levels of triglycerides and free fatty acids. Importantly, most of these beneficial effects were augmented in KO FF+OCA-fed mice ($p < 0.05$).
Conclusions: In conclusion, our results indicate that miR-21 downregulation, likely leading to increased PPARα, together with FXR activation by OCA, ameliorate NAFLD pathogenesis, highlighting the therapeutic potential of novel synergistic therapies in NAFLD. (PTDC/BIM-MEC/0873/2012, SFRH/BD/88212/2012 and SFRH/BD/91119/2012, FCT, Portugal).

P0952

TRANSIENT HEPATIC OVEREXPRESSION OF THE INSULINE-LIKE GROWTH FACTOR 2 (IGF2) INDUCES LIPID DROPLET FORMATION

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Background and Aims: Although insulin-like growth factor 2 (IGF2) has been reported to be overexpressed in steatosis and steatohepatitis, a causal role of IGF2 in steatosis development remains elusive.
Aim of our study was to decipher the role of IGF2 in steatosis development.
Methods: Hydrodynamic gene delivery was used for transient *IGF2* overexpression employing codon-optimized plasmid DNA. Lipids were quantified by vanilloid-sulfuric assay and stained by Scharlach Red. Lipid classes were determined by thin layer chromatography and free cholesterol by filipin staining. Lipid droplets (LD) were evaluated by fluorescence staining with LD540. mRNA and protein levels were quantified by real-time RT-PCR and Western blot, respectively.
Results: Hydrodynamic gene delivery of the IGF2 plasmid resulted in a strong induction of hepatic *IGF2* expression. The exogenously delivered IGF2 had no influence on endogenic *IGF2* expression. The downstream kinase AKT was activated in IGF2 expressing

animals. Analysis of serum parameters revealed decreased ALT levels mirroring the cytoprotective effect of IGF2. Serum cholesterol was increased in IGF2 expressing mice. Histology and a colorimetric assay confirmed lipid accumulation in the IGF2-livers without signs of inflammation. Interestingly, hepatic cholesterol and phospholipids were specifically increased. Phospholipids are mainly incorporated into LD coats and cell membranes. LD size was not changed, but their number was significantly elevated. Furthermore, free cholesterol, which can be stored in LD and has been reported to be critical for steatosis progression, was elevated in IGF2 overexpressing mice. Accordingly, the key enzyme involved in cholesterol biosynthesis, *HmgCoAR* was upregulated. To have a closer look at *de novo* lipid synthesis we investigated expression of the lipogenic transcription factor Srebp1 and its target genes. SREBP1 was induced on both mRNA and protein level. Also Srebp1 target genes were slightly upregulated. Interestingly, the expression of Cpt1a, which is responsible for mitochondrial fatty acid oxidation, was induced.

Conclusions: Hepatic IGF2 expression induces a fatty liver, characterized by increased cholesterol and phospholipids leading to a higher amount of lipid droplets. Therefore, IGF2 might play an important role in the development of steatosis.

P0953

FXR RESISTANCE CHARACTERIZES HUMAN AND MOUSE MODEL OF NASH

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Background and Aims: Non-alcoholic steatohepatitis (NASH) is the most common cause of chronic liver disease in North America and it is commonly associated with type 2 diabetes mellitus (T2DM) and the metabolic syndrome. T2DM is associated with increased bile salts in circulation. Bile salts bind to and activate farnesoid X receptors (FXR) which decrease endogenous bile acids synthesis, decrease gluconeogenesis, enhance triglyceride clearance, inhibit of inflammation and fibrosis. However, these parameters move in the opposite direction in subjects with NASH. To explain this discrepancy, we hypothesized that there is an FXR resistance state in NASH. *Aims:* (1) confirm an increase in plasmatic bile salts in human NASH, (2) test the effects of a diet-induced obesity (DIO) mouse model of NASH that progress to steatosis to steatohepatitis to fibrosis with HCC and fully mimics the human disease.

Methods: Plasma samples were obtained from 7 obese normal, 14 NAFLD and 31 NASH patients and secondary bile salt profiles were measured by GC-MS chromatography. A DIO-mouse model of NASH was also used: male C57BL6/S129 were fed either (1) chow diet with normal tap water or (2) high-fat western diet (WD) with high fructose-glucose solution (HFS) for 8 and 52 weeks. Insulin tolerance tests were performed to evaluate insulin resistance. mRNA expression was assessed using real-time PCR.

Results: As compared to control patients, several circulating secondary bile acids (BA) were increased in NAFLD and NASH patients indicative of a decrease in FXR activation. The mice fed a WD + HFS develop hyperinsulinemia, insulin resistance and hypercholesterolemia and macro- and small droplet steatosis in a centrilobular distribution by week 8, with accompanying inflammation and pericellular fibrosis by week 52. As compared to mice fed a chow diet, mice fed a WD + HFS had a 40% and 80% decrease in mRNA expression for the FXR-target gene small heterodimer partner (SHP) at 8 and 52 weeks respectively. As a direct consequence of decrease expression in the transcription factor SHP, the mRNA expression of the rate-limiting enzyme in BA synthesis cholesterol 7 alpha-hydroxylase (CYP7A1) was increased by 4-fold in mice fed a WD + HFS for 52 weeks. Also, phospholipid transfer protein (PLTP) mRNA was increased by 2-fold and organic anion transporting polypeptides (Oatp-1) mRNA was reduced by 80% in mice fed a WD + HFS for 8 and 52 weeks, both indicative of decreased FXR signalling.

Conclusions: NASH is an FXR resistance syndrome.

P0954

PU-ERH TEA EXTRACT AMELIORATES HIGH-FAT DIET-INDUCED NONALCOHOLIC STEATOHEPATITIS AND INSULIN RESISTANCE BY ENHANCING HEPATIC STAT3 PHOSPHORYLATION IN MICE

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Background and Aims: Pu-erh tea, made from the leaves of *Camellia sinensis*, possess potential health benefits for human, such as anti-inflammatory, anti-oxidant, and anti-obesity properties. Here, we investigated the effect of a Pu-erh tea extract (PTE) on nonalcoholic steatohepatitis (NASH) and its molecular mechanism.

Methods: Eight-week-old male C57BL/6J mice were fed with normal chow diet (ND) or high-fat diet (HFD) for 17 weeks. PTE was simultaneously administered via drinking water for 17 weeks. Body weight and plasma glucose levels were monitored during the treatment. Hepatic inflammation, steatosis, insulin sensitivity, and STAT3 phosphorylation were evaluated. HepG2 cells (human hepatocellular carcinoma cell line) were co-treated with PTE for 48 hours, followed by treatment with IL-6 and oleic acid. The phosphorylation of STAT3, the lipid accumulation, and the expression of lipid metabolism related genes were analyzed.

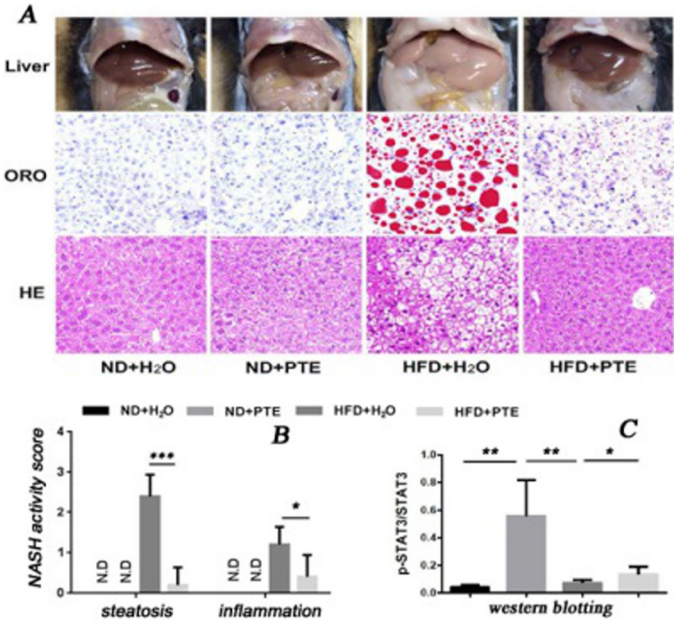

Figure: PTE improves HFD-induced steatohepatitis with enhanced nucleic translocation of STAT3 in hepatocytes. (A) macropathology (upper panels) and Oil Red O staining (middle panels) and HE (lower panels) staining (original magnification ×200). NAS score (B) were calculated. (C) Western blotting analysis were performed to detect the phosphorylated STAT3. ND, normal diet; HFD, high fat diet; H2O, water; PTE, 5% pu-crh tea extract. Data is expressed as the mean ± SEM (n–5). *P < 0.05, **P < 0.01, ***P < 0.001.

Results: PTE inhibited HFD-induced obesity. PTE also significantly attenuated HFD-induced hepatosteatosis and liver inflammation (Fig. A-B). The mRNA expression levels of sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), and pro-inflammatory cytokines were markedly down-regulated in the livers of PTE-treated, HFD-fed mice. Amounts of their lipid droplets were reduced as well. As is expected, glucose and insulin tolerance tests revealed that PTE improved both glucose tolerance and insulin sensitivity. Moreover, insulin receptor substrate 2 (IRS2) expression levels were significantly increased, and the gluconeogenesis related genes such as Forkhead box protein O1 (FOXO1), glucose-6-hosphatasecould (G6Pase), and phosphoenolpyruvate carboxykinasereduce (PCK-1) were significantly dampened in the livers. Strikingly, the PTE treatment enhanced STAT3 phosphorylation in the liver (Fig. C). Consistent with those in vivo study, pre-treatment of HepG2 cells with PTE enhanced IL6-induced STAT3 phosphorylation and attenuated oleic acid-induced lipid accumulation in a manner dependent on STAT3.
Conclusions: These findings indicate that PTE ameliorates the hepatic lipid accumulation, inflammation, and insulin resistance in HFD-fed mice presumably through enhancing hepatic STAT3 phosphorylation in mice.

P0955
COMBINATION THERAPY WITH LOSARTAN AND YC-1 PRODUCES BROWNING PHENOMENON OF WHITE ADIPOSE MASS IN OB/OB MICE

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Background and Aims: Browning process is characterized by promoting activity of mitochondrial uncoupling protein 1 (UCP1) as well an unique functionally thermogenesis in white adipose tissue (WAT), as well as can resist weight gain, obesity and improve metabolic syndrome from obese individuals. Losartan is an angiotensin II receptor antagonist and YC-1, a hypoxia-inducible factor 1 (HIF1) inhibitor, the effect of combine losartan and YC-1 in WAT have never been studied before, therefore we hypothesis that combine therapy with losartan and YC-1 produces browning process in abdomen WAT in *ob/ob* mice.
Methods: Male $Lep^{ob/ob}$ obese mice were administrated with losartan (100 mg/L) for 30 days and YC-1 (25 mg/kg) at last 10 days or vehicle control. Analysis of thermogenic genes (Ucp1 and Prdm16, Cox7a1) expression by real-time PCR from adipose tissue. Hematoxylin/eosin and immunohistochemistry stain of numerous lipid vacuoles, thermogenic factors (UCP1, Tmem26) and inflammatory monocytes (F4/80) were evaluated for histological analysis.
Results: Combination therapy with losartan and YC-1 were markedly ameliorates body weight and abdominal white adipocytes size in ob/ob mice. Treatment of the *ob/ob* mice with losartan only moderately increased Tmem26, Cox7a1 and Prdm16 mRNA expression; however combined treatment with YC-1 enhanced the thermogenic genes and protein expression, subsequently suppressed the induction of inflammation response in neutrophil and marcrophage (F4/80) infiltration expression in WAT.
Conclusions: Combine therapy with losartan and YC-1 show protective role through increased white adipose tissue browning process adaptive the development of metabolic dysfunction, therefore it may illustrate the therapeutic for metabolic syndrome.

P0956
MIR-22 DEFICIENCY EXACERBATES SYSTEMIC AND HEPATIC METABOLIC DISORDERS ASSOCIATED WITH DIET-INDUCED OBESITY IN MICE

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Background and Aims: With obesity and diabetes, miR-22 is drastically downregulated in the liver suggesting an important role for this microRNA in hepatic metabolic disorders. However, direct *in vivo* data examining the role and function of miR-22 in metabolic diseases are lacking. The aim of this study was to investigate *in vivo* the role of miR-22 deficiency in diet-induced obesity, insulin resistance and non-alcoholic fatty liver diseases.
Methods: *MiR-22* knockout (miR22KO) mice were generated and fed an obesogen diet for 4 weeks or 4 months. Mice were metabolically phenotyped and histological and molecular analyses were performed on primary hepatocytes and explanted tissues. High throughput proteomic analysis was performed with liver tissues of CTL and miR22KO mice.
Results: *MiR-22* constitutive deletion in mice breed in normal conditions was completely asymptomatic with no phenotypical changes. However, when mice were fed a high fat-containing diet for a short or long period (4 weeks/months), miR-22 deficiency dramatically exacerbated fat mass gain, glucose intolerance, hepatomegaly and liver steatosis as compared to control mice. Analyses of the liver proteome by mass spectrometry, of *ex-vivo* liver tissues and of *in vitro* primary/cultured hepatocytes indicated that miR-22 inhibition alters the expression of numerous important regulators of the hepatic glucose and lipid metabolism.
Conclusions: miR-22 deficiency in peripheral organs, including the liver, is an important pathological mechanism fostering the development of systemic and hepatic metabolic disorders associated with diet-induced obesity.

P0957
VITAMIN D3 ACTS ON THE GUT–LIVER–ADIPOSE TISSUE AXIS TO MODULATE OBESITY AND ASSOCIATED METABOLIC AND INFLAMMATORY CHANGES IN DIET-INDUCED OBESE MICE

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Background and Aims: Obesity and associated metabolic disorders are increasing problems in industrialized countries. Serum 25-OH Vitamin D3 (VD3) levels negatively correlate with BMI, insulin resistance and NAFLD. The causal relation between VD3 status and NAFLD as well as the potential underlying mechanisms are, however, not fully understood.
Methods: The impact of VD3 on obesity and NAFLD was explored in two animal models. In a first set of experiments, obesity and early NAFLD were induced in wild-type C57BL/6 mice by high-fat diet (HFD)-feeding. HFDs contained predominantly long-chain fatty acids (LCFA) and different levels of VD3 (500 vs. 10,000 IU/kg diet). Liver histology and hepatic fat content were analysed. Expression of metabolic and inflammatory target genes in liver and adipose tissue (AT) was measured by qPCR. Serum parameters were determined by ELISA. To address tissue-specific effects of VD3, a

second set of experiments was performed with Vitamin D Receptor (VDR)-deficient mice (VDR-KO) and VDR-KO mice with an intestine-specific human VDR transgene (VDR-KO hTg) on a similar HFD.
Results: In wild-type mice, high dietary VD3 enhanced the HFD-induced gain of body weight and fat mass. This was associated with unfavourable effects in AT including increased pro-inflammatory M1 macrophage markers and signs of increased adipocyte apoptosis and reduced insulin signalling. These AT-specific changes coincided with trends towards increased immune activation and ER stress in the liver. In the second set of experiments, VDR-KO mice were resistant to HFD-induced obesity when compared to heterozygous controls that is in accordance with previous observations. Interestingly, however, this was partially reversed in VDR-KO hTg mice which gained more weight and fat mass compared to VDR-KOs. Similarly, VDR-KO animals were protected from fatty liver (assessed by histology and mRNA expression) and this effect was partially reversed in the VDR-KO hTg group.
Conclusions: These data reveal a potential pro-adipogenic effect of VD3 in the applied HFD-model based on LCFAs at least during early NAFLD. Comparison of VDR-KO and VDR-KO hTg mice suggests that this could possibly be driven by an unanticipated role of VDR in the regulation of intestinal lipid metabolism. This pro-adipogenic effect of the VD3–VDR axis in the gut could, in turn, promote certain unfavourable metabolic and inflammatory effects in AT and liver highlighting the importance of the gut–liver–adipose tissue axis for the pathophysiology of obesity and NAFLD.

P0958
ANIMAL MODELS OF NON-ALCOHOLIC STEATOHEPATITIS: AN EXTENSIVE SYSTEMATIC REVIEW

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Background and Aims: Animal models are widely used in the study of non-alcoholic fatty liver disease (NAFLD). A range of models have been produced to reflect the spectrum of disease, including non-alcoholic steatohepatitis (NASH) and fibrosis. We performed a comprehensive systematic review to identify which animal models demonstrate: NAFLD, type 1 NASH, type 2 NASH, and HCC.
Methods: MEDLINE search for all articles (in English) including "animal model" and "non-alcoholic steatohepatitis" or "non-alcoholic fatty liver disease". Only primary research papers with animal models were included. Models were described by animal, genetic modifications, diet, and if toxic insults were used. Models were assessed for concordance with features of NAFLD: obesity, insulin resistance, steatosis, portal steatohepatitis, centrolobular steatohepatitis, and development of hepatocellular carcinoma (HCC).
Results: MEDLINE search identified 951 articles, 472 were excluded (230 not relevant, 132 reviews or comments, 70 papers could not be obtained, and 39 had no animal model).
Data was extracted from 479 studies, which used 208 different animal models of NAFLD: 72 dietary, 35 genetic, 11 toxic, 4 offspring, and 86 combination models. The most frequently used models were: high fat diet (HFD) in mice (83/479), HFD in rats (64/479), and methionine-, choline-deficient (MCD) diet in mice (64/479).
129/208 models had evidence of Type 1 ("adult") NASH, with predominance of zone 3 inflammation or panacinar steatohepatitis. An *ad libitum* 'Atherogenic diet' (≈ 1% cholesterol + 0.5% cholate, with or without high fat supplementation) for 6–24 weeks in mice (used by 9/479) most accurately recapitulated the NASH phenotype without use of a genetically modified animal.
Only 1/208 models demonstrated a predominance of portal inflammation. Mice fed an *ad libitum* high-fat, high-fructose diet (~4800 kcal/kg diet) for 16 weeks (used by 4/479 studies) most closely reflects paediatric type 2 NASH, with portal fat infiltration, zone 1 steatohepatitis, and portal fibrosis.
19/208 models demonstrated development of hepatocellular carcinoma.
Conclusions: A 6–24 week atherogenic diet is the most accurate model for type 1 NASH without use of genetic modification. A 16-week high-fat, high-fructose diet in mice most closely reflects paediatric type 2 NASH.

P0959
DIETARY INTERVENTION COMPLETELY REVERSES NON-ALCOHOLIC STEATOHEPATITIS IN OBESE AND INSULIN RESISTANT MICE

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Background and Aims: Life style intervention including dietary intervention is the cornerstone of non-alcoholic steatohepatitis (NASH) treatment, although histological evidence for this approach in humans is rather limited and its impact on hepatic pathways involved in NASH is underreported. This can be explained by the limited duration of human trials, the difficulty of obtaining human liver tissue repetitively and the varying patients' adherence to life style measures, even in the context of clinical trials. The therapeutic potential of the angiotensin receptor type 1 blocker Losartan remains controversial because of varying results in a few animal and human pilot studies. We evaluated the effect of dietary intervention versus Losartan on NASH and associated systemic metabolic features in a representative mouse model.
Methods: Mice with pre-existent NASH, obesity, insulin resistance and hypercholesterolemia (Verbeek *et al.*, *Gut*, 2014) were subjected to dietary intervention (based on a switch from a high fat-high sucrose diet to a normal chow diet) or Losartan administration (30 mg/kg/day). A detailed metabolic profiling was performed.
Results: Dietary intervention normalized obesity, insulin resistance and hypercholesterolemia and remarkably, completely reversed all histological features of pre-existent NASH, including fibrosis. At the hepatic molecular level, dietary intervention targeted fibrogenesis (normalization collagen type I alpha 1, transforming growth factor b1, tissue inhibitor of metalloproteinase 1 mRNA levels), lipid metabolism (normalization fatty acid translocase/CD36, fatty acid transport protein 5, fatty acid synthase mRNA levels) and markers related to mitochondrial function (normalization hepatic ATP content and sirtuin1, tumor necrosis factor-a, uncoupling protein 2 mRNA levels). Dietary intervention abolished p62 accumulation, suggesting a restoration of autophagic flux. On the contrary, Losartan did not affect obesity, insulin resistance, hypercholesterolemia or any histological NASH feature.
Conclusions: Dietary intervention is able to completely reverse NASH in our high fat-high sucrose diet induced mouse model. In order to exploit the maximal therapeutic potential of dietary intervention, improving patient adherence should be a main goal in the care of NASH patients in the coming years. The lack of effective pharmacological alternatives and the additional beneficial effect of dietary intervention on other components of the metabolic syndrome further underpin this strategy.

P0960

A DIET ENRICHED IN PALMITIC AND MYRISTIC ACID CAUSES STEATOHEPATITIS BY INCREASING DE NOVO CERAMIDE SYNTHESIS AND ENDOPLASMIC RETICULUM STRESS

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Background and Aims: Non-alcoholic steatohepatitis (NASH) is characterized by lipid overload and progressive liver injury with inflammation and fibrosis. Palmitic acid (PA) is a saturated fatty acid that induces lipoapoptosis by different mechanisms and fuels ceramide synthesis in the endoplasmic reticulum (ER). Conversion of dihydroceramide into ceramide is done by dihydroceramide desaturase, whose activity increases upon myristoylation at the N-terminus with myristic acid (MA). Since the role of *de novo* ceramide synthesis in PA-mediated lipotoxicity and ER stress in the liver is controversial, our aim was to investigate whether the combination of PA and MA potentiates ceramide synthesis and ER stress and their impact in steatohepatitis *in vivo*.
Methods: C57BL/6 male mice were fed custom-made high fat diets based on PA (30% of Kcal), MA (30% of Kcal) or their combination (PM) (30% of Kcal each) as the only lipid source, complemented with essential fatty acids. Animals weight and ingestion were measured weekly until 6 months, when animals fed PM combination began to die. Animals were euthanized and blood and different tissues collected for serum, histology, qPCR, ceramide and ER stress analysis.
Results: Animals fed PA, MA or their combination did not gain body weight. Interestingly, PM diet induced hepatosplenomegaly and a dramatic adipose tissue reduction. Both PA and especially PM-fed mice had elevated blood serum transaminases and serious liver histological alterations, with necrosis and macrophages infiltration. Moreover, liver cytokines expression increased in mice fed PA and PM diets. Interestingly, neither hyperlipidemia nor steatosis was detected in any of the diets, but a significant increase in liver ceramide was observed, especially in PM-fed animals, which triplicated control levels. Lipid metabolism genes expression showed a preference for ceramide lipogenic genes in PA and PM-fed mice, accompanied by an increase in the fatty acid transporter expression CD36 and a reduction of free fatty acids and triglycerides lipogenic genes. Importantly, ER stress markers such as CHOP, P-PERK, ATF6α, Grp78 or XBP1 were increased in PA and particularly in PM-fed animals, which exhibited 2–4 fold augment.
Conclusions: These results indicate that MA potentiates the lipotoxic effects of PA and their combination increased ceramide synthesis and ER stress leading to steatohepatitis.

P0961

MICROVESICLES AS NOVEL MARKERS IN NON-ALCOHOLIC FATTY LIVER DISEASE PROGRESSION

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver pathology and is the most complex to diagnose due to the lack of a clinically useful circulating biomarker. Microvesicles are a potential novel candidate. These submicron vesicles are shed from cells in both physiological and disease conditions, but their secretion profile in NAFLD progression have not been well documented. We therefore investigated the effect of high-fat diet as a model of NAFLD on circulating and liver secreted microvesicles. Additionally we examined their expression of CD147, a proinflammatory molecule known to be secreted in microvesicles and linked to dysregulated tissue remodelling and hepatic fibrogenesis.
Methods: Six week old male C57Bl/6 mice were fed a high-fat diet (45%kcal fat) or chow *ad libidum* for 12 or 50 weeks. At termination, blood and livers were harvested for circulating and secreted microvesicles respectively. These were isolated by ultracentrifugation of platelet-poor plasma or serum-free media collected from 18 hr culture of liver sections. Microvesicle concentration and size were quantified using NanoSight™ while CD147 protein expression was determined by immunoblotting.
Results: Circulating microvesicle numbers were elevated in steatotic animals versus controls for both timepoints (by 2.2 and 12.6-fold, $P < 0.01$). In contrast, we observed a significant decline in the corresponding liver secretome per gram tissue (by 2.5 and 25.9-fold, $P < 0.02$). The microvesicle size distribution was unchanged. Immunoblotting revealed distinct changes in the liver microvesicle protein signature between the timepoints which also translated to CD147 expression; the high glycan form of the molecule was significantly reduced with high-fat feeding at 12 weeks (63% decrease, $P < 0.02$) and was almost completely diminished by 50 weeks.
Conclusions: These results show dysregulation between circulating and tissue microvesicle numbers which was more prominent at 50 weeks, the more physiologically advanced stage of NAFLD. The proportional reduction in microvesicular CD147 secreted from the liver is an interesting phenomenon that could be explained by either the retention of the molecule within the liver during steatosis, or an alternate pathway for its translocation. Together these findings suggest that circulating microvesicles have potential utility as biomarkers of NAFLD.

P0962

MANIFESTATION OF DIET-ASSOCIATED NAFLD/AFLD IN DIFFERENT MOUSE STRAINS

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Background and Aims: Animal models are essential for the investigation of non-alcoholic and alcoholic fatty liver disease (NAFLD and AFLD, respectively). Still the complex pathophysiological mechanisms contributing to NAFLD/AFLD pathogenesis are incompletely understood. The aim of our study was to compare the manifestation of liver damage caused by different feeding regimens in commonly used wild type (wt) mouse strains in order to identify those best suited for modelling human NAFLD/AFLD.
Methods: Accordingly, 3 different wt mouse strains (C57BL/6, CD-1 and 129Sv) were fed with liquid Lieber DeCarli high fat diet (HFD), liquid Lieber DeCarli diet supplemented with alcohol and liquid Lieber DeCarli HFD supplemented with alcohol for different periods of time. The effects on liver histology as well as serum markers of liver function (aspartate-aminotransferase, AST and alanine-aminotransferase, ALT) were assessed.
Results: C57BL/6 mice responded very variable to the feeding regimens. Some of these mice featured pronounced steatosis, liver inflammation as well as elevated AST and ALT serum levels, whereas other animals showed no liver damage. No statistical difference between treated mice and controls with respect to histological or biochemical features was observed. CD-1 mice did not develop significant steatosis or inflammation and their AST/ALT serum levels were not different to controls. Most consistent results were achieved with 129Sv mice, which consistently developed moderate steatosis in combination with inflammation. Histological features of steatosis and inflammation and AST serum levels were

significantly higher in HFD and alcohol feeding groups compared to controls (Kruskal–Wallis followed by Dunn's test of selected pairs of columns, $P < 0.05$), while no significant differences in ALT levels could be detected.

Conclusions: As a conclusion, our data suggest the use of 129Sv mice for NAFLD/AFLD studies, in which a homogeneous response to feeding regimens is needed. If more pronounced liver injuries are required, C57BL/6 mice should be preferred. But a higher number of animals will be needed, since not all mice will develop liver damage. The use of CD-1 mice is discouraged because of the lack of histological and biochemical response to standard dietary regimens, used to induce NAFLD/AFLD-associated liver damage.

P0963

PROGRESSION FROM NAFLD TO NASH: GENDER DOES REALLY MATTER?

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Background and Aims: NAFLD is most prevalent among males, bur NASH has been reported more prevalent among middle-aged women. The aim of this study is to assess the events involved in the progression of the disease in both genders.

Methods: Male and female C57BL/6J mice, immediately after weaning were randomly assigned to either control diet (CTRL) or high-fat high-carbohydrate (HFHCD). Animals had ad-libitum access to these diets for 16 weeks, thereafter blood and liver samples were collected for further analysis (histology, gene expression and products of lipoperoxidation). Data was compared *vs* animals of each gender fed with CTRL diet.

Table 1. Summary of the obtained data

	Folds vs animals fed control diet, mean±SD	
	HFHCD males	HFHCD females
Body weight	1.43±0.15***	1.46±0.13***
Liver weight	1.70±0.20***	1.50±0.12***
Adipose tissue weight (epididymal fat pads)	3.12±0.40***	3.20±0.34***
Serum parameters		
Glucose	1.65±0.29***	1.64±0.19***
Insulin	7.30±4.30**	1.75±0.30
Cholesterol	1.50±0.17***	7.3±4.3***
HDL	1.52±0.14***	1.52±0.24***
LDL	1.70±0.20***	2.40±0.50***
ALT	3.15±0.88**	1.88±0.65*
AST	2.30±0.33**	1.75±0.27
Gene expression		
Lipid metabolism		
DGAT2	1.64±0.31*	2.25±0.06***
LDLR	2.09±0.51*	1.72±0.08*
SREBP-1c	1.35±0.16*	1.65±0.31*
Inflammatory response		
TNF-α	2.16±0.94*	1.13±0.25
Fibrosis		
α-SMA	2.03±0.48*	2.28±0.37*
Lipoperoxidation		
MDA	0.87±0.29	3.82±0.40***

*p < 0.05; **p < 0.01; ***p < 0.001.

Results: HFHCD induced weight gain, hyperplasia of adipose tissue, hepatomegaly, alterations in glycaemia, lipid profile and ALT both in males and females; hyperinsulinaemia was present exclusively in males (Table 1). Histological analysis showed mixed macro-micro vesicular steatosis in both genders, in line with the increased expression of DGAT2 (important in the final step of triglycerides synthesis), LDLR, receptor and SREBP-1c, both involved in the lipogenesis. Likewise, both groups developed sinusoidal/periportal fibrosis with increased activation of hepatic stellate cells (α-SMA). Surprisingly, only males showed inflammatory cells foci with some ballooning and increased expression of TNF-α, whereas females presented only an increased lipid peroxidation (absent in males) with no signs of inflammation.

Conclusions: Altogether these data suggest that even if the onset of fibrosis is similar between genders, the mechanism of liver injury is different. Whereas in males is associated to insulin resistance and inflammation, in females is related with an increase of the oxidative process.

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P0964

DIRECT GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISM AMELIORATES STEATOHEPATITIS AND FIBROSIS IN MODELS OF NASH AND BILIARY FIBROSIS VIA REGULATION OF LIVER MACROPHAGE INFILTRATION AND ACTIVATION

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Background and Aims: Glucagon-like peptide-1 (GLP-1) improves insulin sensitivity via enhanced glucose-dependent insulin secretion, inhibition of glucagon release, and delayed gastric emptying following its release into the circulation from the gut. We aimed to explore the utility of the long-acting GLP-1 receptor agonist BYDUREON (BY) to address both inflammation and fibrosis in models of NASH and biliary fibrosis.

Methods: BY was administered twice weekly by subcutaneous injection at 0.4 or 2 mg/kg to Mdr2KO mice, and to C57BL/6 mice fed a methionine and choline deficient (MCD) diet for 4 weeks. Hepatic fibrosis was assessed by morphometric analysis of Sirius red stained collagen and measurement of hydroxyproline content. Hepatic inflammation was measured by semiquantitative immunohistochemistry. Fibrosis and inflammation related transcript levels were quantified by quantitative real-time polymerase chain reaction (qPCR). Ex vivo analysis ofhepatic inflammatory cells was performed by FACS

Results: In the MCD and Mdr2$^{-/-}$ model, BY significantly decreased collagen content, fibrosis and inflammation related transcripts and protein levels including αSMA, CD68, CCL3, and TNFα, while it increased the (anti-inflammatory) macrophage markers Arg1and Ym1. BY treatment also reduced the IHC expression of procollagen type III, CD68, F4/80 and caspase 3. With BY treatment the number of liver infiltrating CD45$^+$CD11b$^+$ (myeloid, innate) immune cells and of F4/80$^+$ Kupffer cells was reduced. With By treatment there was a significant decrease of hepatic CD11b$^+$Ly6C$^+$ high cells, indicating less proinflammatory monocyte/macrophage infiltration as compared to mice that received the MCD diet without BY. Differences were even more pronounced for the ratio of CD11b$^+$Ly6C$^+$low or CD11b$^+$Ly6C$^+$ high vs CD45$^+$ cells. BY treatment also significantly lowered elevated serum liver enzymes.

Conclusions: Treatment of MCD diet treated and of Mdr2KO mice with BY reduced parameters of hepatic steatosis, inflammation, fibrosis and apoptosis, without negative effects on weight gain, liver or plasma indices. These results demonstrate that treatment with BY attenuated liver injury through inhibition of inflammatory and apoptosis pathways that govern NASH and fibrosis and support further clinical evaluation of the utility of GLP-1R agonists for treatment of NASH and liver fibrosis.

P0965

HIGH FAT DIET TREATMENT OF CONDITIONAL c-met KO-MICE DEVELOP AN INFLAMMATORY PHENOTYPE AND PROGRESS TO FIBROSIS INDEPENDENTLY FROM STEATOSIS

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Background and Aims: The metabolic syndrome including non-alcoholic-fatty liver disease (NAFLD) turns out to be one of the fastest growing medical problems in industrial countries. NAFLD progressing to NASH (non-alcoholic steatohepatitis), can ultimately result in liver cirrhosis and hepatocellular carcinoma. Previously we could show that HGF/c-Met signalling protects against the development of NASH in a Methionine-Choline Deficient Diet mouse model. Now we aimed to characterize the role of HGF/c-met in a more clinically relevant model of the metabolic syndrome
Methods: Hepatocyte specific c-met knockout mice (c-met$^{\Delta hepa}$) using the cre-loxP system and wildtype control mice (c-met^{loxPloxP}) were fed with a High Fat Diet (HFD) at least for 16 weeks.
Results: After 16 weeks of HFD feeding c-met^{loxPloxP} mice show a significant higher liver weight and more epididymal fat compared to c-met$^{\Delta hepa}$ animals. Mice lacking c-Met display a significantly decreased liver body weight ratio, while both groups presented microvesicular steatosis in hepatic HE- and Oil Red O Stainings and a similar increase of hepatic triglyceride content. Furthermore both groups showed increased blood glucose level under diet conditions, but c-Met$^{\Delta hepa}$ littermates presented significantly lower levels of hepatic Glucose-6-Phosphatase-RNA, a key-enzyme of hepatic gluconeogenesis after a 16 week HFD treatment. Moreover, c-Met knockout mice displayed a significant increase of hepatic PPAR-y RNA levels as compared to c-met^{loxPloxP} mice after 16 weeks of HFD treatment. Most interestingly we could detect a significant increase of infiltrating immune cells in c-Met$^{\Delta hepa}$ mice compared to wildtype animals (in particular neutrophils, macrophages and CD4 positive T cells). Further analyses provide evidence for a beginning liver remodelling in c-Met$^{\Delta hepa}$ littermates as indicated by an upregulation of hepatic Col1a1 and TGF-beta-RNA. Those data were corroborated by higher collagen deposition and alpha-SMA staining.
Conclusions: Hepatocyte specific deletion of c-Met causes the development of a distinctive hepatic inflammation and matrix deposition process under the condition of HFD-treatment. Those changes were largely independent from the development of liver steatosis.

P0966

ADIPOSE TISSUE INFLAMMATION OCCURS PRIOR TO LIVER INFLAMMATION IN MICE FED A HIGH-FAT DIET

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Background and Aims: Chronic metabolic inflammation is considered to be important in the etiology of obesity-induced insulin resistance. However, the extent to which inflammation in the adipose tissue and the liver contributes to insulin resistance remains unknown. Here we examine the sequence of inflammatory events between adipose tissue and the liver and their relevant contribution to the development of insulin resistance.
Methods: C56BL/6J male mice fed a low-fat diet (LFD; 10% kcal fat) or high-fat diet (HFD; 45% kcal fat) for either 24, 40 or 52 weeks. Glucose tolerance and plasma levels of glucose, insulin, leptin and adiponectin were measured throughout the study. Adipose and hepatic tissues dissected to assess lipid accumulation and inflammation.
Results: With HFD, mice developed a progressive obesity, evidenced by increased body weight and increased lipid accumulation in adipose and liver tissues. This phenotype was accompanied by the development of insulin resistance after 24 and 40 weeks of HFD, but insulin resistance was attenuated after 52 weeks of HFD. We observed inflammation of the adipose tissue, as measured by an increase in crown-like structures and upregulation of pro-inflammatory genes *Tnf, Il1*b, *Mcp1 and F4/80* after 24 weeks of HFD and made similar observations in the liver only after 40 weeks of HFD.
Conclusions: Our data show that adipose tissue inflammation precedes liver inflammation. Moreover, insulin resistance is present both in the absence (24 weeks of HFD) and in the presence (40 weeks of HFD) of hepatic inflammation. We conclude that obesity-related insulin resistance is more likely to be caused by adipose tissue inflammation than by liver inflammation.

P0967

ROLE OF FATTY ACIDS FOR CHOLANGIOCYTE LIPID METABOLISM AND LIPOTOXICITY

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Background and Aims: Impaired free fatty acid (FA) handling results in hepatic lipotoxicity. Saturated FAs including palmitate (PA) are poorly incorporated into triglycerides resulting in endoplasmic reticulum (ER) stress and lipoapoptosis. Conversely, monounsaturated FAs such as oleate (OA) reduce lipotoxicity. Excess of FAs has been linked to hepatocellular injury; but little is known about their impact on cholangiocytes with potential relevance for cholangiopathies and progression of fatty liver disease.
Aims: To determine the capacity of cholangiocytes to handle FA-mediated stress and decipher the underlying molecular mechanisms.
Methods: Mouse cholangiocytes were incubated with OA and PA (200–800 μM 24 hrs). Lipid droplets were measured using Oil Red O staining (ORO). Cell death, apoptotic markers and reactive oxygen species (ROS) were quantified. Expression of key genes involved on lipid metabolism was analyzed by QPCR.
Results: Cholangiocytes expressed enzymes involved in FA synthesis (FASn), oxidation (CPT1A) and transport (CD36). PA but not OA induced cell death, as shown by viability assay ($p < 0.005$); whereas OA supplementation rescued PA toxicity ($p < 0.05$). Lipotoxicity induced by PA was associated with ER stress markers such as GRP78, sXBP-1, ERDJ4 mRNA ($p < 0.05$; $p < 0.005$ and $p < 0.005$ respectively), while OA co-incubation normalized the ER stress response. This resulted in pro-apoptotic signaling as shown by CHOP mRNA induction and caspase 3 cleavage ($p < 0.05$ and $p < 0.005$). OA addition reduced apoptosis ($p < 0.05$). OA increased lipid storage (ORO) and reduced free FAs in cholangiocytes ($p < 0.05$), as well as PPARγ2 and DGAT gene expression (both $p < 0.05$). FA oxidation was significantly increased by PA compared to control and OA, as shown by elevated CPT1A gene expression ($p < 0.05$). Remarkably, PA+OA incubation increased the expression of antioxidative genes such as catalase and SOD

(both $p < 0.005$) resulting in ROS elevation only in PA treated cells, as revealed by dihydroethidium staining and i-NOS expression. Notably, the anti-oxidant N-acetylcysteine counteracted PA induced lipoapotosis as shown by viability assays ($p < 0.005$).
Conclusions: Saturated FAs induced ER stress, oxidative stress and apoptosis in cholangiocytes. Importantly, lipotoxicity can be counteracted by stimulating ROS detoxification and FA storage.

P0968
ARE THE TYPE OF PROBIOTIC STRAINS AND THEIR AMOUNT EQUALLY EFFECTIVE FOR NAFLD PREVENTION? EXPERIMENTAL COMPARATIVE STUDY

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Background and Aims: Today probiotics have been suggested as a treatment for the prevention of chronic liver damage, because they prevent bacterial translocation and epithelial invasion, inhibit bacterial mucosal adherence, production of antimicrobial peptides, that results in decrease of inflammation and stimulation of host immunity. However the question about the comparison of efficacy of different probiotic strains, their combination and form (alive or lyophilized) in management of nonalcoholic fatty liver disease (NAFLD) is still open.
Methods: We included 70 rats divided into 7 groups 10 animals in each. Rats of group I were intact. Newborn rats of groups II–VII were injected with monosodium glutamate (MSG) (4 mg/g). The groups III–V received lyophilized monoprobiotics *B. animalis VKL*, *B. animalis VKB*, *L. casei IMVB-7280* respectively. The group VI received the mix of these three probiotic strains. The group VII was treated with multiprobiotic "Symbiter" containing concentrated biomass of 14 alive probiotic bacteria (*Bifidobacterium, Lactobacillus, Lactococcus, Propionibacterium).* Administration was started at 4 weeks after birth and continued intermittently two-week course in 2 weeks intervals. To assess morphological changes in liver we used NAS (NAFLD activity score). Lipid extraction from liver was performed according to Folch.
Results: For steatosis stage there was no significant difference between MSG-obesity group and lyophilized groups III–VI strains. But we found significantly lower degree of steatosis (2.3 ± 0.21 vs 0.7 ± 0.15, $p < 0.001$) for alive probiotic group (VII) as compared to MSG-obesity. For both alive and lyophilized probiotic mixtures reduction of lobular inflammation was observed. These histological data were confirmed by the significant decrease of total lipids and triglycerides content in liver approximately by 22–25% in groups treated with probiotic mixtures (VI, VII) compared to MSG-obesity.
Conclusions: Thus, the obtained data suggest failure of NAFLD prevention with lyophilized monoprobiotic strains. Opposite we established the efficacy of probiotic mixture with the preference of alive probiotic strains. It may be related to more pronounced viability of alive strains, their prevention of bacterial translocation, formation of mutualistic interactions in mixtures and therefore synergistic enhancement of single effect.

P0969
ROLE OF EPIGENETIC FACTORS IN NON-ALCOHOLIC STEATOHEPATITIS DEVELOPMENT

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Background and Aims: To evaluate micro-RNAs influence in non-alcoholic steatohepatitis (NASH) development.
Methods: Total RNA was isolated from frozen hepatic tissue (miRvana miRNA isolation kit, Life Technologies, USA) in 10 NASH and 10 simple steatosis patients. RNA Integrity Number (RIN) was measured by electrophoresis as quality control. Subjects presenting a RIN <5 were not suitable for analysis. miRNA profile was assessed using the 84-miRNA miScript PCR Array (Qiagen, USA). Independent validation was performed in liver biopsy ($n = 20$) and plasma samples ($n = 40$, 20 NASH and 20 simple steatosis) by RT-PCR. Two SNPs were genotyped: PNPLA3 rs738409 and TM6SF2 rs58542926 by Taqman assay (Applied Byosistems, Spain). Liver histology was evaluated by Kleiner score. Statistical analysis was performed by SSPSv22.
Results: In screening, two miRNAs were found over-expressed in NASH vs simple steatosis: (a) miR-200b (fold induction 2.80); (b) miR-224 (fold induction 3.08).
In liver tissue ($n = 16$), 100% (8/8) of patients with *miR-200b* upregulated presented NASH (5.91 ± 8.70) vs 0% (0/8) with miR-200b inhibited (-2.53 ± 0.61; $p = 0.0001$). miR-200b induction was associated with ballooning [62.5% (5/8) vs. 0% (0/8); $p = 0.026$]. 100% (5/5) of patients with *miR-224* upregulated presented NASH (3.95 ± 2.05) vs 9% (1/11) with miR-224 inhibited (-3.46 ± 1.87; $p < 0.0001$). Over-expression of miR-224 was associated with NAS Score >4 [60% (3/5) vs 0% (0/11); $p = 0.018$], steatosis presence [100% (5/5) vs 27% (3/11); $p = 0.026$] and portal inflammation [60% (3/5) vs 0% (0/11); $p = 0.018$].
In plasma ($n = 22$), *miR-200b* induction was associated with NASH [91.7% (11/12) *vs.* 50% (5/10); $p = 0.05$] and ballooning [66.7% (8/12) vs. 10% (1/10); $p = 0.011$] in patients with miR-200b inhibited. *miR-224* was found upregulated in 100% (9/9) of NASH patients. In patients protected from NASH development, such as non-GG PNPLA3 carriers, miR-224 induction was detected in 6/9 NASH vs 0/7 with simple steatosis; $p < 0.01$; also in patients bearing TM6SF2 CC genotype, miR-224 induction was observed in 7/9 of NASH vs 0/6 with simple steatosis; $p = 0.007$.
Conclusions: miRNA profile analysis associated to hepatic dysfunction has identified two candidates, miR-200b and miR-224, overexpressed in NASH at intrahepatic level and circulating in plasma. miR-224 expression seems to regulate NASH progression in patients bearing protective genotypes, as non-GG PNPLA3 and CC TM6SF2. These miRNAs could constitute potential biomarkers or novel therapeutic targets based on its marked epigenetic effect.

P0970
FAT-LADEN MACROPHAGES MODULATE LOBULAR INFLAMMATION IN NONALCOHOLIC STEATOHEPATITIS (NASH)

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Background and Aims: NASH is characterized by extensive hepatic monocyte infiltration and macrophages have an important role in regulating the disease evolution. However, little is known about the functional changes occurring in liver macrophages during NASH progression. In this study we have investigated phenotypic and

functional modifications of hepatic macrophages in experimental NASH.
Methods: NASH was induced in C57BL/6 mice by feeding a methionine-choline deficient (MCD) diet up to 8 weeks.
Results: In mice receiving the MCD diet hepatic F4/80-positive macrophages increased in parallel with the disease progression. Histology revealed that the macrophages accumulating in advanced NASH were enlarged, vacuolized and formed small aggregates. At immunofluorescence these cells contained lipid vesicles positive for the apoptotic cell markers Annexin V suggesting the phagocytosis of apoptotic bodies derived from dyeing fat-laden hepatocytes. Similar vacuolized macrophages were also evident in liver biopsies of NASH patients. Flow cytometry revealed that these enlarged macrophages expressed the inflammatory monocyte markers (CD11b, Ly6C). However, as compared to regular size macrophages the enlarged sub-set was characterized by a lower IL-12 production and by an enhanced expression of the anti-inflammatory mediators IL-10 and annexin A1. Consistently, the accumulation of enlarged macrophages in NASH paralleled with a lowering in the expression of the macrophage M1 activation markers iNOS and IL-12, while the levels of M2 polarization markers arginase-1 and MGL-1 were unchanged.
Conclusions: Altogether, these data indicate that during the progression of NASH the phagocytosis of hepatocyte-derived apoptotic bodies promotes functional changes in macrophages that influence hepatic inflammatory responses.
This work has been supported by a grant from the Fondazione Cariplo, Milan, Italy.

P0971
A DIET-INDUCED MOUSE MODEL OF NONALCOHOLIC FATTY LIVER DISEASE WITH PROGRESSION TO ADVANCED FIBROSIS AND HEPATOCELLULAR CARCINOMA WITH A GENE EXPRESSION SIGNATURE MIMICKING HUMAN DISEASE
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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) has become the most common form of chronic liver disease and has the propensity to progress to cirrhosis and hepatocellular cancer (HCC). Currently no validated animal model of NASH exists. We have recently described a diet induced obesity (DIO) model of NAFLD in mice devoid of genetic or chemical manipulation that progressively develops steatosis, inflammation, hepatocyte ballooning, fibrosis and HCC. To compare the gene expression profile of this mouse model to human NASH and HCC and to compare histologic scoring of this model over time.
Methods: A unique isogenic mouse strain derived from a C57Bl/6J and 129Sl/SvlmJ background was created. Mice were fed one of two diets: (1) chow diet with normal tap water, (2) high-fat diet (HFD) + high fructose–glucose solution (HFS). Histology was assessed from hematoxylin+eosin and sirius red. Histologic scoring using the NASH Activity Score (NAS) and Fibrosis scoring was done at early and late time points. Liver tissue was snap-frozen for analyses. Liver tissue was compared between diets at early and late time points using genome wide expression profiles and this data was compared to human disease. Furthermore, mouse HCC found at 52 weeks on the HFD+HFS, was compared to human HCC using Gene Set Enrichment Analysis (GSEA). Normalized enrichment scores (NES) were used to compare mouse gene expression to human disease.
Results: Compared to chow diet, HFD+HFS fed mice had larger, pale livers with the addition of numerous tumors in the livers at 52 weeks. Macro- and small droplet steatosis in a centrilobular distribution developed universally in HFD+HFS mice by 8 weeks with accompanying inflammation and some hepatocyte ballooning. By week 36–52, there was increasing fibrosis with some bridging fibrosis, hepatocyte ballooning and Mallory bodies. 0/15 mice fed CD developed HCC by wk 52 compared to 8/9 mice fed HFD+HFS ($p < 0.01$). The HFD+HFS fed mice showed a gene signature similar to that expressed in humans with a NES of 1.81, $p < 0.01$, and a False discovery rate (FDR) <0.01. Furthermore, the signature of the HCC tumors in HFD+HFS fed mice were similar to aggressive human HCC of the S1 (NES = 1.46, $p < 0.01$, FDR = 0.013) and S2 (NES = 1.42, $p < 0.01$, FDR = 0.014) molecular classifications. Wnt signaling pathways were also increased in in the mouse HCC as in human S1 HCC.
Conclusions: This DIO model of NASH and HCC in mice has a gene expression signature similar to that seen in humans with advanced NASH and HCC.

P0972
EXPRESSIONS OF BILE ACID TRANSPORTERS ARE INVERSELY CORRELATED WITH NAFLD ACTIVITY SCORE IN THE LIVER OF PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE
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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is increasing in the world including Asian countries. NAFLD includes a disease spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). The latter is considered as a progressive disease and its pathogenesis remains largely unclear. Recently, bile acid (BA) metabolism is focused as a therapeutic target of NASH. The aim of this study was to identify changes of bile acid metabolism in NAFLD patients in terms of disease progression.
Methods: Fifty male and thirty-three female patients histologically diagnosed as NAFLD by Matteoni classification were analyzed. Patients taking UDCA were excluded in this study. Disease progression was estimated by NAFLD activity score (NAS). Intrahepatic expression levels of genes related to BA metabolism were determined by quantitative PCR.
Results: FXR, the nuclear receptor for BA, and its downstream transcriptional repressor SHP mRNA levels were not significantly changed in both genders. CYP7A1, a key enzyme of BA synthesis, was also similarly expressed. However, expressions of an export transporter, bile salt export pump (BSEP), and an uptake transporter, NTCP, were significantly down-regulated with the elevation of NAS in male. In female, interestingly MRP2, another BA export transporter and NTCP were significantly down-regulated with the elevation of NAS.
Conclusions: Expressions of BA uptake and export transporters are significantly down-regulated in patients with high NAS in both genders. The BA content of hepatocyte is thought to be increased in NASH. The down-regulation of NTCP is reasonable for the protection from BA cytotoxcicity. But, the down-regulation of BSEP and MRP2 may cause the excess of BA content in hepatocyte and excessive BA may induce hepatocellular injuries. Although the mechanism of the down-regulation and the difference between genders remain to be elucidated, our findings might lead to the development of new therapeutic options for NASH.

P0973

QUERCETIN AMELIORATES MCD-INDUCED NON-ALCOHOLIC FATTY LIVER DISEASE IN MICE BY MODULATING INFLAMMATORY, OXIDATIVE/NITROSATIVE STRESS AND LIPID METABOLISM-RELATED GENE DEREGULATION VIA THE PI3K/AKT PATHWAY

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Background and Aims: Flavonoids seem to display beneficial effects on non-alcoholic fatty liver disease (NAFLD). In this study, we aimed to investigate the effect of the flanovol quercetin on gene expression deregulation involved in NAFLD development, as well as the possible implication of phosphatidylinositol 3-kinase (PI3K)/AKT pathway modulation.
Methods: C57BL/6J male mice were fed with methionine and choline deficient (MCD) diet supplemented or not with quercetin (0.05%) for 4 weeks (1W-4W). Huh7 cells were incubated for 24 hours with MCD medium supplemented with or without quercetin (10 μM) and/or the PI3K inhibitor LY294002 (50 μM). ROS and RNS production, lipoperoxidation and lipid accumulation were assessed by flow cytometry. Fatty acid uptake and FAT/CD36 localization were analysed by fluorescence microscopy. Lipid metabolism, pro-inflammatory and oxidative/nitrosative-related gene expression was measured by RT-qPCR. PI3K/AKT pathway activation was assessed by western blot.
Results: MCD-fed mice showed classical pathophysiological characteristics of non-alcoholic steatohepatitis (NASH), with increased lipoperoxidation, inflammation and lipid accumulation, associated with gene expression deregulation (TNFα, 3W: +412%; SOCS3, 3W: +61%; OPN, 4W: +152%; iNOS, 3W: +308%; FAT/CD36, 4W: +5800%; FABP1, 3W: −72%; FATP5, 3W: −74%; FOXA1, 4W: −70%; PPARα, 3W: −39%; C/EBPα, 4W: −48%; SHP, 3W: −79%; C/EBPβ, 4W: +238%, *vs* control mice). MCD-incubated Huh7 cells showed similar gene expression deregulation. PI3K/AKT pathway was activated by MCD (MCD-fed mice, 4W: +169%; MCD-treated Huh7 cells: +208%, *vs* respective controls) and triggered gene deregulation causing either activation or inhibition of studied genes as demonstrated through cell incubation with LY294002 (FAT/CD36: −76%; FABP1: −17%; SOCS3: −61%; TNFα: −95%; FOXA1: −29%; SHP: −71%; C/EBPβ: −67%; iNOS: +132%; FATP5: +168%; PPARα: +430%; C/EBPα: +63%, *vs* MCD-treated cells). Treatment with quercetin reduced AKT phosphorylation and oxidative/nitrosative stress, inflammation and lipid metabolism-related genes displayed a tendency to normalize in both *in vivo* and *in vitro* models.
Conclusions: These results place quercetin as a potential therapeutic strategy for preventing NAFLD progression by attenuating gene expression deregulation, at least in part through PI3K/AKT pathway inactivation.
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P0974

EFFECTS OF RESVERATROL ON EXPERIMENTAL NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: Non-alcoholic steatohepatitis is an increasing clinical problem for which effective treatments are required. The polyphenol resveratrol prevents the development of experimental non-alcoholic steatosis in numerous *in vivo* studies due to anti-inflammatory, anti-oxidant and AMPK/SIRT1 activating effects. We hypothesized that resveratrol would reverse steatohepatitis, including hepatic inflammation and fibrosis, in a well-characterised rodent model.
Methods: To induce steatohepatitis, a 65% fat, 2% cholesterol (HFC) diet was fed to rats for 1 or 16 weeks, prior to treatment. Subsequently, the diet was supplemented with resveratrol (100 mg/rat/day) to 3 intervention groups, week 2–5, 2–8, and 17–23. Treated animals were sacrificed at the end of each intervention period with appropriate control and HFC diet controls. Blood and liver were harvested for analysis.
Results: Resveratrol treatment partially mitigated hepatic enlargement and transaminase elevations when commenced early, but not plasma bilirubin or α2-MG elevations. Resveratrol treatment did not modidy the elevated hepatic triglyceride levels or histological steatohepatitis. We observed a minor reduction in Coll1α1 mRNA in intervention group 17–23 ($p = 0.03$) and generally no significant alterations in the mRNA expression of TGFβ, TNFα, α2-MG, PGC-1α, SREBP-1c. Protein expression of TNFα induced protein-3 was lowered by *trans*-resveratrol ($p = 0.03$).
Ten resveratrol metabolites, including *trans*-resveratrol-3-*O*-sulphate and *trans*-resveratrol-4′-*O*-sulphate, were quantified in plasma by LC-MS/MS at a total mean concentration of 24.7 μg/ml.
Conclusions: Contrary to the findings in experimental steatosis, resveratrol treatment had only limited therapeutic efficacy in alleviating experimental steatohepatitis.

P0975

CHARACTERIZATION OF AN IN VIVO MODEL OF JUVENILE NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: The booming increase worldwide prevalence of pediatric Non-Alcoholic Fatty Liver Disease (NAFLD) is worrisome and draws attention.
Methods: Male (M) and female (F) C57BL/6J mice were randomly assigned immediately after weaning to control or high-fat high-carbohydrate diet (HFHCD). Animals had access ad-libitum to food for 16weeks. Body-weight, glycaemia, insulinemia, triglycerides, total cholesterol, HDL-C, ALT, AST, liver histology were screened every 4 weeks and compared to control animals (CTRL).
Results: Soon after the first week, HFHCD induced in both genders a significant gain of bodyweight. Males, after 4 weeks presented

Table 1 (abstract P0975). Summary of the obtained data both in male and female

Parameter	Folds vs CTRL, mean±SD							
	Male				Female			
	4 weeks	8 weeks	12 weeks	16 weeks	4 weeks	8 weeks	12 weeks	16 weeks
Body weight	1.15±0.06***	1.3±0.1***	1.43±0.14***	1.43±0.15***	1.17±0.05***	1.17±0.07***	1.29±0.12***	1.46±0.13***
Liver weight	1.43±0.28	1.1±0.18	1.6±0.19*	1.7±0.2***	0.85±0.33	1.09±0.007	0.7±0.2	1.5±0.12***
Fat tissue weight (epididymal fat pads)	1.91±0.26*	4.7±0.57**	3.74±0.22***	3.12±0.40***	1.13±0.26	1.60±0.8	1.7±0.7	3.2±0.34***
Glucose	1.18±0.08*	1.16±0.1*	1.45±0.1***	1.65±0.29***	1.25±0.17*	1.29±0.21**	1.48±0.15**	1.64±0.19***
Insulin	2.1±0.3**	2.06±1.2*	4.4±4.3	7.3±4.3**	1.07±0.15	1.0±0.1	0.88±0.3	1.75±0.3
Cholesterol	1.47±0.09**	2.66±0.19**	1.96±0.17**	1.5±0.17***	1.26±0.04*	0.95±0.01	1.2±0.14	1.48±0.2***
HDL	1.5±0.004***	2.58±0.16**	1.9±0.30*	1.52±0.14***	1.35±0.06*	1.05±0.01	1.5±0.24	1.52±0.24***
LDL	2.03±0.06***	4.64±0.12***	3.09±0.38**	1.76±0.25***	1.88±0.08***	1.10±0.03	1.50±0.28	2.46±0.5***
ALT	1.68±0.36*	1.15±0.09	4.45±0.55**	3.15±0.88**	0.6±0.12	0.98±0.07	1.31±0.29	1.88±0.65*
AST	1.28±0.19	1.27±0.38	2.51±0.38*	2.30±0.33**	1.00±0.40	0.88±0.25	1.55±0.30	1.75±0.27

*p < 0.05; **p < 0.01; ***p < 0.001.

also hyperplasia of epididymal fat-pads and only after week 12th, a significant hepatomegaly. As shown in Table 1, males showed earlier alteration of glycemia, insulinemia as well as lipid profile and ALT. Interestingly, comparable body/blood alterations were observed in females only at the 16th week. Liver histology showed in both genders a mixed macro-microvesicular steatosis steadily increasing after the 8th week. Inflammatory cells foci were observed in males from the very beginning and sustained over the time with presence of ballooning cells towards the 16th week. On the contrary this inflammatory pattern was absent in females. Surprisingly, both genders developed progressive fibrosis starting from the 8th week and rising steadily over the time

Conclusions: This juvenile model of diet-induced NAFLD progresses faster than those reported in adults. A clear gender difference was found in the onset of the liver injury. Even though the final outcome (fibrosis) was comparable between genders, males presented early signs of liver injury, while females do not.

P0976

EFFECT OF ROSUVASTATIN OR/AND β-CAROTENE AND DIETARY CONTROL ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN RATS

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Background and Aims: The prevalence of non-alcoholic fatty liver disease (NAFLD) has markedly increased, especially with patient exhibit one or more features of the metabolic syndrome. This study investigates the effect of rosuvastatin (RSV) or/and b-carotene (bC) in NAFLD-induced rats.

Methods: Rats were classified into nine groups; normal (I), NAFLD-induced with high-fat diet (HFD; II), NAFLD switched to regular diet (RD; III), NAFLD-HFD or NAFLD-RD treated with RSV (IV, V), bC (VI, VII) or both RSV+bC (VIII, IX) respectively for four weeks then killed to obtain serum samples and liver tissues. Liver histology, lipid profile, liver oxidative stress markers, and adipocytokines were measured.

Results: Liver sections of NAFLD-HFD rats revealed steatosis, loss of hepatic architecture, inflammation and hepatocyte vacuolation with high percentage of cell fibrosis. Serum levels of ALT, AST, ALP, GGT and lipid profile (triglycerides, cholesterol, LDL and VLDL) were significantly increased ($P < 0.05$) compared with normal. Also, hepatic MDA level and serum leptin, TNF-a and TGF-b1 were increased. Meanwhile, SOD activity, GSH content and serum HDL and adiponectin were decreased ($P < 0.05$) *vs* normal. These changes were to a less extent in NAFLD-RD group. Administration of RSV or/and bC almost improves all previously mentioned parameters. Moreover, hepatic steatosis was decreased and inflammation markedly ameliorated with reduction of TNF-a and TGF-b. These results were more pronounced in the groups VIII and IX *vs* each drug alone.

Conclusions: RSV and βC could be beneficial for the treatment and prevention of NAFLD. Combined RSV+bC is better than RSV alone.

P0977

ASSOCIATION OF LONG-CHAIN FATTY ACID OXIDATION WITH OXIDATIVE STRESS AND INFLAMMATION IN DIFFERENT PREECLAMPSIA-LIKE MOUSE MODELS

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Background and Aims: The pregnancy complication of preeclampsia as a syndrome has been further recognized. The long-chain fatty acid oxidation (FAO) disorders may be associated with some preeclampsia, but the mechanism of FAO in the pathogenesis of subset of preeclampsia syndrome remains unclear. This study aimed to explore the role of FAO and its relationship with oxidative stress and inflammatory in preeclampsia syndrome by establishing four different preeclampsia-like models.

Methods: PE-like groups were ApoC3 transgenic mice with abnormal fatty acid metabolism, classical PE-like models with injection of Nw-nitro-L-arginine-methyl ester (L-NA) or lipopolysaccharide (LPS) and the antiphospholipid syndrome (APS) mouse model with beta2GPI injection (ApoC3+NS, ApoC3+L-NA, L-NA, LPS and beta2GPI groups). The control group was wild-type mice with normal saline injection. Serum FFA concentration and the mRNA and protein expression of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), p47phox and NF-kB in placental and hepatic cell were detected. The correlation was analyzed statistically.

Results: Compared with controls, FFA levels were significantly increased in all groups except in the LPS group ($P < 0.05$). LCHAD mRNA and protein expression in the liver and placenta significantly increased in ApoC3+NS, ApoC3+L-NA and beta 2GPI group, decreased in L-NA group ($P < 0.05$) and had no significant difference in the LPS group compared to the control group. P47phox and NF-kB mRNA and protein expression in the liver and placenta displayed variations according to different preeclampsia-like models. Serum FFA levels positively correlated with P47phox and NF-kB mRNA and protein expression in liver of all groups ($P < 0.05$) except in LPS group and in placenta of L-NA and beta2GPI groups ($P < 0.05$).

Conclusions: The liver and placenta may be more crucial sites of production of toxic intermediates of long-chain free fatty acid metabolism, which accumulate to cause liver damage in subset preeclampsia syndrome. There is different interaction of long-chain

FAO disorders with oxidative stress and inflammation in subset pre-eclampsia syndrome.
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Fatty liver disease: b. Clinical

P0978
A 7 DAY LOW VS HIGH GLYCAEMIC INDEX DIET REDUCES LIVER FAT CONTENT IN HEALTHY SUBJECTS: A ^{1}H MRS STUDY

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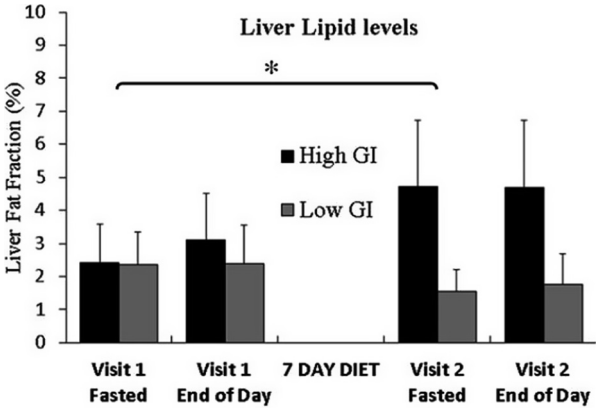
Background and Aims: Glycaemic index (GI) is a way of ranking carbohydrates according to the postprandial effect on blood glucose levels and has received increasing interest in recent years. Low glycaemic index diets have been considered potentially beneficial in a range of metabolic conditions and cohort studies have shown correlations between dietary glycaemic index and non-alcoholic fatty liver disease (NAFLD). The aim of this study was to investigate the effects of a low (LGI) v high (HGI) glycaemic index diet on hepatic lipid stores.
Methods: ^{1}H Magnetic resonance spectroscopy (MRS) was used to assess liver lipid levels before and after a 7 day HGI v LGI dietary intervention in a two-way randomized cross over study with >4 week washout between test visits. 8 healthy males (age 20.1±0.4 years, BMI 23.0±0.9 kg/m^2) attended the test centre and blood glucose measurements were taken at baseline and regularly following a HGI v LGI test breakfast. MRS was acquired at baseline and end-of-day (t = 360 min) on a Philips 3T scanner using a respiratory triggered PRESS localisation sequence with varying TE for T_2 correction (BW = 2 kHz, samples = 1024, TR = 5000 ms, NSA = 40, TE = 40 ms, 50 ms, 60 ms, 80 ms). Areas under the CH_2 (1.3 ppm) and water (4.7 ppm) peaks were used to calculate tissue weight fat fraction.



Results: Blood samples confirmed a high and low glycaemic response for respective test meals. On the pre diet test day, fasted liver fat fractions were consistent for both HGI and LGI arms (HGI 2.4±1.2%; LGI 2.4±1.0%; P = 0.82) and end of day lipid levels were within error of fasted levels. Following the 7 day diet, fasted liver fat fractions increased for HGI (4.7±2.0%) compared with LGI (1.6±0.7%) reaching statistical significance (two way F-test, P < 0.05) and this effect was consistent across the test day.
Conclusions: This study used a simple MRS protocol to show the beneficial impact on liver lipid levels of a LGI compared with HGI diet after only 7 days. This has important applications in the design of dietary interventions to prevent and treat NAFLD as well as its metabolic consequences. Further studies should explore longer term interventions and patient groups.
Acknowledgements: We wish to thank Unilever and BBSRC for funding this study.

P0979
PILOT STUDY OF A NEW TREATMENT IN NAFLD/NASH, INTERFERING INTESTINAL MICROBIOTA AND BILE ACIDS RESORPTION AND METABOLISM

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Background and Aims: Intestinal microbiota plays an important role, modulating inflammation and absorption of bile acids, thus its dysfunction might trigger the NAFLD/NASH. Rifaximine (RFX) is known to eradicate intestinal bacterial overgrowth, followed by a symbiotic (SYN) that increases colonization with Bifidobacteria, exert an immunomodulatory effect, induction of tall-like receptors, increase of IL10 production and inhibition of Th1 lymphocyte generation. Ursodeoxycholic acid (UDCA) also, has been used in previous studies in NASH. *Aim:* Assess the efficacy of a new combined therapy consisting in RFX, followed by SYN formula and associated to UDCA in a discontinued regimen for improving NASH.
Methods: 40 patients diagnosed with NASH on transaminases elevation, serological tests for NASH/fibrosis, associated diseases and liver histology, were prospectively randomized between January 2012 and September 2014 into 2 similar groups: 20 patients were treated with RFX 1200 mg/day 10 days, followed by SYN 1 bag/day, 10 days, in association with UDCA 15 mg/kg/day; 20 patients received only UDCA 15 mg/kg/day. This regimen was repeated for 3 consecutive months, 3 times/year, with final evaluation after 1 year.
Results: Both groups were comparable: mean age 45–47 years; more men, 12/20 and 14/20; 16/20 in group 1 and 15/20 in group 2 were overweight, 90% with abdominal obesity; 40% group 1 and 50% group 2 had diabetes mellitus, 50% with insulin resistance; 80%, respectively 70% had hyperlipidemia; 30% vs. 50% had HTA. 90% over 80% of patients had AST/ALT ratio >1, with an increased level by 1.5×. NASH test was positive in all patients, on liver biopsies steatosis was over 60%, inflammation grade 2 to 3 and fibrosis grade 2, no liver cirrhosis. In patients treated with the combined regimen, no side effects were reported, with an improvement in bloating, abdominal pain, and diarrhea/constipation symptoms. Associated diet, change in life style, medication to correct the co-morbidities had a compliance of about 60%. After 1 year of follow-up improvements were found with regard to: weight loss of 4–10% in 70% of cases in both groups, normalization of ALT, AST and insulin sensitivity in 90% of patients from group 1, vs. 40% in group 2, decrease in steatosis infiltration by 40% and in fibrosis by 1 stage only in group 1 with combined treatment.
Conclusions: Several cycles of an associated regimen with RFX+SYN+UDCA, improved significantly liver histology, steatosis and inflammation in about 60% of patients with NASH.

P0980

DIET, ENDOTOXAEMIA AND FATTY LIVER: A POPULATION STUDY USING MAGNETIC RESONANCE SPECTROSCOPY AND TRANSIENT ELASTOGRAPHY IN 920 CHINESE SUBJECTS

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Background and Aims: Patients with non-alcoholic steatohepatitis (NASH) have increased intestinal permeability and small intestine bacterial overgrowth. We aimed to test the hypothesis that endotoxaemia is associated with fatty liver in the general population and to study dietary factors associated with endotoxaemia.
Methods: Community adult subjects were randomly selected from the Hong Kong Government's census database and underwent proton-magnetic resonance spectroscopy and transient elastography to assess hepatic steatosis and fibrosis, respectively. Intrahepatic triglyceride content (IHTG) of 5% was the cutoff to define fatty liver. Endotoxaemia was assessed using the Limulus Amebocyte Lysate, lipopolysaccharide-binding protein (LBP) and EndoCab immunoglobulin G (IgG) assays. Dietary pattern was recorded by a 7-day food-frequency questionnaire.
Results: 920 subjects were included (42% male, age 48±11, 23% body mass index ≥25 kg/m^2 and 5% diabetes). 263 (29%) had fatty liver; 60 (7%) had raised serum cytokeratin-18 fragment level (CK-18) suggestive of NASH; 27 of 887 (3%) subjects with reliable liver stiffness measurement had advanced fibrosis or cirrhosis. Compared with those without fatty liver, subjects with fatty liver had higher LBP (13.4±3.2 μg/ml vs 11.4±2.7 μg/ml; $P < 0.001$) and EndoCab IgG (228±247 GMU/ml vs 188±137 GMU/ml; $P = 0.013$) levels. Endotoxin markers also correlated positively with aminotransferases, IHTG, CK-18, insulin resistance and dyslipidaemia. Endotoxaemia was not associated with increased liver stiffness. Fetuin-A, the ligand linking fatty acid and Toll-like receptor 4, correlated with IHTG, insulin resistance and dyslipidaemia but did not have consistent association with endotoxaemia markers. Although total energy consumption and individual macronutrients were not associated with endotoxaemia, current drinkers (median alcohol consumption 20 g/week [interquartile range 10–70 g]) had lower endotoxin, EndoCab IgG and fetuin-A levels than non-drinkers.
Conclusions: Endotoxaemia is associated with fatty liver and possibly NASH in the general population. People with modest alcohol consumption have lower serum endotoxin. This may partly explain the lower risk of fatty liver and NASH in modest drinkers in previous observational studies.
This study was supported by a grant from the Health and Medical Research Fund sponsored by the Hong Kong SAR Government [Ref CUHK-11120621].

P0981

ADAPTATION OF HEPATIC MITOCHONDRIAL FUNCTION IN OBESE HUMANS WITH OR WITHOUT NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: Lower muscle mitochondrial activity relates to insulin resistance and non-alcoholic fatty liver (NAFL). However, hepatic mitochondrial function in insulin resistant humans with NAFL or steatohepatitis (NASH) remains unclear. Here we applied high-resolution respirometry (HRR) to quantify mitochondrial respiration in liver biopsies of obese insulin resistant humans with histologically-proven NAFL and NASH.
Methods: We performed HRR in liver tissue and isolated mitochondria to assess *ex vivo* maximal coupled and uncoupled respiration in obese humans with NAFL ($n = 16$, age 41±3 years, body mass index, BMI 53.7±2.1 kg/m^2) and obese humans with NASH ($n = 7$, age 51±3 years, BMI 47.3±0.7 kg/m^2) undergoing bariatric surgery, as well as in lean humans undergoing elective abdominal surgery (CON; $n = 12$, age 41±3 years, BMI 25.5±0.7 kg/m^2). Whole-body and hepatic insulin-sensitivity measured with euglycemic-hyperinsulinemic clamps and [6,6-$^2H_2$]glucose. Citrate synthase activity served as a marker of hepatic mitochondrial mass. Hepatic oxidative stress and oxidative damage were assessed from thiobarbituric acid reactive substances and hepatic 8-OH-deoxyguanosine (8-OH-dOG), respectively.
Results: Both NAFL and NASH displayed markedly lower whole-body insulin sensitivity than CON (M-value in mg/kg·min; NAFL 2.5±0.3, NASH 1.4±0.9, CON 7.7±0.8, $p < 0.05$), whereas only NASH had lower hepatic insulin sensitivity. In the face of similar mitochondrial content, NAFL presented with 84% higher maximal uncoupled respiration in isolated mitochondria than CON, whereas NASH had 40% lower respiration rates than NAFL despite compensational increase of mitochondrial mass. Both NAFL and NASH showed evidence of mitochondrial uncoupling, but only NASH exhibited increased proton leakage. Hepatic oxidative stress was elevated in both NAFL and NASH, while oxidative DNA damage was noted only in NASH (8-OH-dOG in nM; NAFL 18.5±4.5 vs. CON 11.3±0.9, $p < 0.05$) due to reduced anti-oxidant defense capacity (hepatic catalase activity in arbitrary units; NAFL 0.67±0.09, NASH 0.36±0.20, CON 0.86±0.08, $p < 0.05$).
Conclusions: These data provide direct evidence for adaptation of liver mitochondria in obese insulin resistant humans with NAFL. In livers of NASH patients, this adaptation is lost, which associates with hepatic insulin resistance, leaking mitochondria and hepatocellular oxidative damage along with impaired anti-oxidant defense.

P0982

NONALCOHOLIC FATTY LIVER DISEASE AND THE RISK OF PROGRESSED CORONARY ARTERY CALCIFICATION: A LONGITUDINAL STUDY

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is associated with coronary artery calcification and increased prevalence of cardiovascular disease in many cross-sectional studies. However, whether NAFLD *per se* affect the progression of coronary artery calcification still remains to be elucidated. The aim of this study was to investigate the prospective association between NAFLD and the incidence and progression of coronary artery calcification (CAC) in a longitudinal cohort study.
Methods: Among 1732 subjects who underwent serial CAC score evaluation, we evaluated 847 subjects with NAFLD and 885 subjects without NAFLD. Only those without viral hepatitis, significant alcohol consumption and known coronary artery disease were enrolled. NAFLD was diagnosed by ultrasonography in subjects without significant alcohol consumption. CAC score was evaluated by the Agatston method with multi-detector computed tomography.
Results: The baseline CAC score was higher in those with NAFLD, and greater number of these subjects showed progression (48.8% vs. 38.4%, $p < 0.001$ in subjects with vs. without NAFLD). NAFLD was prospectively associated with progression of CAC score (odds ratio [OR] 1.53, 95% confidence interval [CI] 1.26–1.85, $p < 0.001$). Noticeably, the impact of NAFLD significantly varied with the severity of baseline coronary atherosclerosis. In those without calcification at baseline, NAFLD significantly affected progression of atherosclerosis (adjusted OR 1.54, 95% CI 1.05–2.27, $p = 0.028$), after adjustment for age, hypertension diabetes mellitus, dyslipidemia, smoking, gender and body mass index. Analysis according to the severity of NAFLD showed that NAFLD in its more severe form promotes progression of CAC (adjusted OR 1.77, 95% CI 1.08–2.88, $p = 0.022$). However, in subjects with baseline CAC, NAFLD did not affect progression of CAC ($p = 0.482$).
Conclusions: NAFLD plays a role in early stage of coronary atherosclerosis, and it also seems that greater degree of NAFLD affects the progression independent of traditional risk factors. Thus NAFLD does not solely show a simple association, but also plays a role in the development and progression of coronary artery disease.

P0983

ASSOCIATION BETWEEN MACRO-NUTRIENT INTAKE AND PRESENCE OF NONALCOHOLIC FATTY LIVER DISEASE IN THE ROTTERDAM STUDY: A POPULATION-BASED STUDY

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Background and Aims: Specific macronutrient intake may be associated with presence of nonalcoholic fatty liver disease (NAFLD).
Methods: Macronutrient intake was assessed by the validated Food Frequency Questionaire in participants of the Rotterdam study, a large population-based cohort study of Caucasian subjects aged ≥55 years. In this cohort, liver steatosis was assessed by ultrasound and liver fibrosis was assessed by transient elastography. All participants with reliable liver stiffness measurements (LSM) between January 2010 and March 2014 were included in the analyses. Subjects were excluded in case of viral hepatitis, excessive alcohol intake or hepatotoxic medication or if the LSM suggested significant fibrosis (LSM >8.0 kPa), in the absence of steatosis on ultrasound. Three separate univariate logistic regression analyses were performed to assess the effect of different macronutrients on the odds of having NAFLD. All analyses were corrected for total energy intake.
Results: 1357 subjects were included. In 903 subjects there were no signs of either steatosis or fibrosis and 454 subjects (33%) had non-alcoholic fatty liver disease i.e. steatosis with or without fibrosis.
Total carbohydrate intake (OR 0.997, 95% CI 0.994–0.999, p = 0.010), total protein intake (OR 1.010, 95% CI 1.002–1.018, p = 0.010) and total fat intake (OR 1.006, 95% CI 1.001–1.011, p = 0.023) were all associated with presence of NAFLD, whereas total fiber intake was not (OR 1.000, 95% CI 0.985–1.015, p = 0.954).
Conclusions: Our study suggests that higher intake of protein and fat are associated with increased odds of NAFLD.

P0984

DEVELOPMENT AND VALIDATION OF A SIMPLE CLINICAL PREDICTION MODEL TO IDENTIFY PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS RESOLUTION AFTER 52 WEEKS OF LIFESTYLE MODIFICATION

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Background and Aims: The identification of predictors of nonalcoholic steatohepatitis (NASH) resolution after therapeutic intervention are need to avoid unnecessary liver biopsies. Thus, we developed and validated a simple scoring system including clinical, laboratory and histological variables to predict NASH resolution after 52 weeks of lifestyle intervention.
Methods: A total of 293 patients with histological diagnosis of definite NASH were treated in the clinical practice with low-fat hypocaloric diet and increased physical activity for 52 weeks. At baseline, patients were randomly stratified into two cohorts to develop (199) and validate (94) a predictive model. Subjects with NASH borderline and cirrhosis were excluded. A second liver biopsy was performed at 52 weeks to detect NASH resolution with no fibrosis impairment. Liver biopsies were reviewed by two pathologist who were unaware of patient's identity and clinical condition. Clinical, histological and laboratory data were recorded at baseline and at the end of the intervention.
Results: NASH resolution occurred in 31% (derivation group) and 34% (validation group). In the derivation cohort, weight loss percentage (OR, 2.03; 95% CI, 1.5–2.7; P < 0.0001), type 2 diabetes mellitus (OR, 0.05; 95% CI, 0.009–0.26; P < 0.001), ballooning (OR, 0.15; 95% CI, 0.04–0.59; P = 0.001), ASAT (OR, 0.96; 95% CI, 0.93–0.99; P = 0.002), and body mass index (OR, 0.83; 95% CI, 0.72–0.93; P = 0.01) were independently associated to NASH resolution. The area under the ROC curve (AUC) of the selected model was 0.9297 (95% CI, 0.896–0.962) and 0.883 (95% CI, 0.817–0.950) in the derivation and validation cohorts, respectively. Using a score threshold of <−0.705 corresponding with a low probability of NASH resolution, negative predictive values were 93% and 91% in the derivation and validation groups, respectively. By applying a high cutoff >1.418 which corresponds with high probability of NASH resolution, positive predictive values were 97% and 85% in the derivation and validation groups, respectively. Using both cutoffs, a liver biopsy would have been avoided in 223 (76%) of 293 patients with a correct prediction in 203 (91%).

Conclusions: A simple prediction model may help to identify patients with low and high probabilities of NASH resolution after 52 weeks of lifestyle modification. Unnecessary liver biopsies could be avoided using our proposed score.

P0985

METABOLOMICS PROFILING IDENTIFIES POTENTIAL PATHWAYS INVOLVED IN THE INTERACTION OF IRON HOMEOSTASIS WITH INSULIN RESISTANCE

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Background and Aims: Elevated serum ferritin has been linked to type 2 diabetes and adverse outcomes in subjects with the Metabolic Syndrome (MetS). In NAFLD subjects mild iron overload is frequently found and has been named dysmetabolic iron overload. As the mechanisms underlying the negative impact of excess iron have so far remained elusive, we aimed to identify potential pathways using a metabolomics analysis.
Methods: In all subjects, a detailed clinical and biochemical characterization was obtained. Metabolomics profiling was performed in patients with NAFLD with (n = 56) and without iron overload (n = 54) and a lean, healthy control group (n = 53) utilizing the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences AG). Significant differences between groups were calculated using the false discovery rate (FDR) approach. NAFLD was diagnosed as steatosis on ultrasound examination after biochemical exclusion of other causes of liver disease.
Results: Clinically, subjects with NAFLD and high ferritin had higher indices of impaired glucose homeostasis as assessed by fasting glucose, HOMA-IR and oral glucose tolerance test ($P < 0.01$, Man-Withney-U test) compared to NAFLD subjects matched for components of the MetS without iron overload. Comparison of the metabolome between NAFLD subjects and healthy controls confirmed results from previous studies, i.e. differences in concentrations of branched-chain amino acids, kynurenin, and multiple long-chain lyso-phosphatidylcholines (FDR <0.001 for all analytes). In addition, the comparison between NAFLD with high and low ferritin demonstrated highly significant differences in the serum concentrations of sarcosine, methionine, and citrulline as well as multiple long-chain phosphatidylcholines (FDR <0.001 for all analytes).
Conclusions: Our data confirm that in NAFLD high serum ferritin concentrations are clinically linked to impaired glucose homeostasis as indicated by oral glucose tolerance test and fasting glucose concentrations. Furthermore, our study identifies novel associations of iron excess in NAFLD subjects with distinct subsets of phosphatidylcholines as well as a pathway involving methionine, sarcosine and citrulline. These metabolic pathways may be involved in iron-induced augmentation of IR.

P0986

EFFECTS OF SHORT TERM VERY LOW ENERGY DIETS PRIOR TO BARIATRIC SURGERY ON LIVER HISTOLOGY AND CIRCULATING BIOMARKERS: RESULTS OF A RANDOMISED CONTROLLED TRIAL (RCT)

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Background and Aims: Short term very low energy dietary regimens are used prior to bariatric surgery with the aim of reducing liver volume and improving liver flexibility to enable the laparoscopic approach to surgery. We examined the effects of very low energy dietary regimes on non-alcoholic fatty liver disease (NAFLD) and circulating biomarkers in severely obese adults.
Methods: The effects of two energy restrictive regimens, a food-based diet or meal replacement plan (LighterLife Ltd., Harlow, U.K.) over a two week period, were investigated in a prospective RCT. Clinical and anthropometric information and fasting blood were collected pre and post diet, and a liver biopsy obtained during surgery.
Results: Of 60 participants recruited 54 completed the study. Diet groups did not differ demographically, 81.5% female, median age 45 years, median body mass index (BMI) pre-diet 50.7 kg/m^2, 30% with Type 2 diabetes. Reported daily energy intake and body weight loss were similar in both groups (median 715kcal/d and median 3.4% respectively). Liver histology was not significantly different between groups; 86% had steatosis grade ≤1, 71% had no ballooning and 61% were without lobular inflammation. Serum leptin, IL6 and CRP fell in both groups. Changes in other biomarkers are in Table 1. A fall in Enhanced Liver Fibrosis (ELF) panel markers (pre to post diet) were associated with increased body weight loss (%) ($p = 0.05$). ELF levels post-diet were positively associated with steatosis (%) ($p = 0.01$). Also ELF levels post-diet above the median were associated with higher frequency and values of NAFLD activity scores ($p = 0.001$).

Table 1

Biomarker	Pre–post diet change			
	Food based diet		Meal plan	
	Change, median (range)	P value	Change, median (range)	P value
Aspartate transaminase	7.0 (−3.0, 22.6)	<0.001	5.5 (−7.6, 40.3)	<0.001
Cytokeratin-18 M30	15.9 (−119, 209)	0.019	20.2 (−94.1, 228)	0.011
Enhanced liver fibrosis (ELF) panel	−0.6 (−2.3, 0.6)	0.001	−0.6 (−1.6, 0.7)	0.002

Conclusions: Two weeks of energy restrictive diet prior to bariatric surgery were associated with a reduction in serum leptin, IL6 and CRP and an increase in AST and CK18 M30. On liver biopsy low prevalence of advanced liver histological changes were found, contemporaneous to a fall in circulating ELF panel markers. No statistically significant difference in histological outcomes was found between diet groups. Overall, short-term very low energy dietary regimes positively affect NAFLD.

P0987

A METABOLIC DEFINITION OF NASH AND ITS RESOLUTION BY S-ADENOSYLMETHIONINE IN Mat1a KO MICE

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Background and Aims: *Mat1a* KO mice spontaneously develop nonalcoholic steatohepatitis (NASH), which highlights the role of this gene in hepatic energy stores. *MAT1A* encodes the enzyme that catalyzes the conversion of methionine into S-adenosylmethionine (SAMe), the main biological methyl donor. NASH patients often have reduced expression of *MAT1A*, indicating that the *Mat1a* KO mouse model not only recapitulates the histological and metabolic features of NASH but it is also a relevant model to study human NASH.
Methods: Here we used NASH *Mat1a* KO mice (showing ultrasound fatty liver and elevated ALT) to: (1) evaluate if oral SAMe treatment for two months reverts NASH; (2) study the hepatic metabolic phenotype of NASH in *Mat1a* KO mice treated with placebo or SAMe (over 500 metabolites were analyzed); and (3) obtain a lipid signature of NASH through the serum lipidomic phenotype in *Mat1a* KO mice. To translate these findings to humans, we examined the presence of this identified lipid signature of NASH in *Mat1a* KO mouse in the serum of patients with biopsy proven NASH diagnosis.
Results: (1) The histological exam of liver samples revealed a marked reduction of steatosis, inflammation, necrosis and fibrosis as well as a normalization of serum ALT after SAMe treatment in *Mat1a* KO mice. (2) Lipidomic studies show that the main effect of SAMe in NASH treatment is to improve mitochondrial function, which leads to the reestablishment of a normal response to the accumulation of hepatic fatty acids (FA) (increased FA β-oxidation and decreased lipogenesis) and to the reduction of FA and triglyceride content. In addition to this, SAMe reestablished normal bile acid metabolism. (3) We compare the serum lipidomic profile in WT and *Mat1a* KO mice to generate a serum signature of NASH comprised of 50 metabolites (M-type signature). To translate these findings to humans, we carried out a hierarchical clustering of the serum lipidomic data of 134 patients with biopsy-confirmed NASH. Patients were classified into two well-defined clusters based on optimum average silhouette width: one cluster with an M-type signature that included 78 patients (58.2%) and a second cluster that contained 56 patients (41.8%), which suggests that the target for NASH patients with an M-type signature may be considered when assessing the efficacy of SAMe treatment.
Conclusions: These results show that SAMe treatment halted progression of NASH and reverted toward normal histology in *Mat1a* KO mice.

P0988

NONALCOHOLIC FATTY LIVER DISEASE: SERUM BILE ACID LEVELS IN CHILDREN AND ADOLESCENTS AS MARKER FOR PROGRESSION?

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is frequent in obese children. A reliable non-invasive biomarker for monitoring of progression to liver fibrosis would be useful. Serum bile acid (BA) levels are elevated in cirrhosis, probably for mechanical reasons. Interestingly, BA can influence glucose and lipid metabolism by stimulating insulin release via the TGR5/GLP1 pathway; and, reciprocally, insulin can downregulate BA synthesis from cholesterol via the FXR/SHP and/or the PI3K/AKT pathways. We hypothesized that changes in BA levels in NAFLD vary depending on grade of fibrosis.
Methods: In this multicenter study adolescents with NAFLD (n = 92) were classified between stages of fibrosis (non-fibrosis n = 27; fibrosis ≥1 n = 65) based on liver-biopsy findings. Metabolic and cholestatic status was assessed by blood tests (glucose, insulin, cholesterol, LDL, HDL, AST, bilirubin, ALT, GGT). The full BA pool, including 15 BA species, was measured by HPLC-MS/MS and compared to healthy controls (n = 105).
Results: Both groups showed hyperglycemia (non-fibrosis 126±44; fibrosis 119±18 mg/dl), hyperinsulinism (83±33 vs 88±41 μE/ml), and elevated ALT levels (63±20 vs 87±58 U/l). Non-fibrotic adolescents had significantly ($p < 0.001$) decreased median BA levels (1.28; range 1.18–2.34 μmol/l) compared to controls (3.36; range 2.16–4.69 μmol/l). In fibrosis BA values increased (1.86; 1.05–3.22 μmol/l; $p < 0.001$). Non-fibrotic patients lacked glycine-conjugated BA with a significant ($p < 0.05$) predominance of unconjugated BA. In fibrosis, glycine-conjugated BA values rose and the BA pool resembled that in healthy controls. Other values did not differ significantly between the groups.
Conclusions: BA levels decrease in early NAFLD and seem to increase continuously during progression to fibrosis and cirrhosis. BA may serve as a non-invasive biomarker for progression of disease.

P0989

A COMBINATION OF ELASTOMETRY AND BLOOD MARKERS IMPROVES THE NON-INVASIVE STAGING OF LIVER FIBROSIS IN NAFLD

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Background and Aims: Accurate evaluation of liver fibrosis is required in NAFLD as patients with advanced (F3/4) fibrosis have an impaired liver-related prognosis. Combining liver stiffness measurement (LSM) by Fibroscan with blood fibrosis tests significantly improves their diagnostic accuracy in chronic hepatitis C, but this combinative tactic remains poorly evaluated in NAFLD. We directly compared LSM and 8 blood fibrosis tests in NAFLD, and developed a new test combination to improve the diagnostic accuracy.
Methods: 646 NAFLD patients with liver biopsy (NASH-CRN F fibrosis stage), LSM, and 8 blood fibrosis tests designed either for NAFLD (BARD, NAFLD Fibrosis Score, FibroMeter[S]) or chronic

viral hepatitis (APRI, FIB4, Fibrotest, Hepascore, FibroMeter^{V2G}) were included.

Results: LSM (0.834±0.014) and FibroMeter^{V2G} (0.798±0.016, p = 0.063 vs LSM) had higher Obuchowski indexes than the 7 other blood tests (p ≤ 0.036). The rate of patients with a reliable diagnosis (≥90% predictive values) of advanced fibrosis was the highest with LSM and FibroMeter^{V2G} (respectively, 50.7% and 46.7%, p < 0.001 vs other fibrosis tests). Independent predictors of advanced fibrosis (LSM median, alpha2-macroglobulin, hyaluronate, prothrombin time, diabetes, AST) were combined in a new BLEN-test. In a validation set, the BLEN-test had a significantly higher Obuchowski index (0.854±0.020) than FibroMeter^{V2G} or LSM (p ≤ 0.015). The rate of patients with a reliable diagnosis (≥90% predictive values) of advanced fibrosis was 74.3% with the BLEN-test (p < 0.001 vs FibroMeterV^{2G} or LSM). Fibrosis classifications derived from LSM or BLEN-test provided 7 fibrosis classes and avoided any biopsy requirement. In the validation set, 85.5% of F3/4 patients were well-classified with the BLEN-test classification vs 69.7% with the LSM classification (p = 0.004). Calculating the BLEN-test with the M or XL probe LSM results did not lead to significant change in the accuracy of the BLEN-test classification.

Conclusions: By comparing 9 non-invasive fibrosis tests in a large population, we identified FibroMeter^{V2G} and Fibroscan as the best tools for liver fibrosis evaluation in NAFLD patients. A combination of Fibroscan result with blood markers significantly improved diagnostic accuracy compared to single fibrosis tests. This combination accurately identified the subset of F3/4 NAFLD patients without any liver biopsy requirement.

P0990

NON-ALCOHOLIC STEATOHEPATITIS IS AN INDEPENDENT PREDICTOR OF MORTALITY IN A COHORT OF 493 MORBIDLY OBESE PATIENTS UNDERGOING BARIATRIC SURGERY

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Background and Aims: The role of liver biopsy in the context of bariatric surgery remains unknown. We aimed to study the prognostic relevance of clinical parameters, including liver histology, on overall survival in a single centre cohort of obese patients undergoing bariatric surgery.

Methods: We included all patients undergoing bariatric surgery in our institution between January 1997 to December 2004 who had routine perioperative liver biopsy to assess for evidence of NAFLD or other liver disease. All had Roux-en-Y gastric bypass. Baseline clinical data were obtained and patient files and public records were checked to assess for mortality. All liver histology specimens were reviewed by an experienced pathologist (LRB) using the NASH Clinical Research Network Scoring System.

Continuous values are described as mean (±SD) and categorical values as n (%). Survival analysis was performed using the log-rank test and Cox-proportional hazards.

Results: 500 patients underwent gastric bypass surgery of whom 493 (99%) had a histological sample for analysis. Baseline characteristics were: male gender 91 (18%), age 41 y (±10), BMI 45 kg/m^2 (±6.7), AST 23 IU/ml (±14), ALT 34 IU/ml (±26), total cholesterol 5.3 mmol/L (±1.2), triglycerides 1.7 mmol/L (±1.2), HOMA-IR score 7.5 (±7.6), diabetes 82 (16%), arterial hypertension 156 (32%). Baseline histology demonstrated 436 (88%) patients with steatosis >5%, 57 (12%) patients with NASH of which 45 (79%) had fibrosis.

NASH patients were older (46 vs 40 y, p < 0.001), had higher ALT (52 vs 32 IU/ml, p < 0.001), triglycerides (2.2 vs 1.6 mmol/L, p = 0.01) and HOMA-IR (12 vs 7, p = 0.004), were more likely to be diabetic (46 vs 13%, p < 0.001) and to have arterial hypertension (51 vs 29%, p = 0.001) than non-NASH individuals. However, baseline BMI was not statistically different (46 vs 45, p = 0.49) between the 2 groups.

Twenty-one patients (4.2%) died during a mean follow-up of 9.4 y (±3.9). Univariate and multivariate survival analyses are shown in Table 1. Baseline factors independently associated with mortality were arterial hypertension and presence of NASH. Fibrosis was not included in the multivariate analysis due to multi-collinearity with NASH.

Table 1. Baseline factors associated with overall mortality in survival analysis

Characteristic	Survival group		Univariate analysis		Multivariate analysis	
	Alive	Dead	HR	p-value	Adjusted HR	p-value
Age	41 y	47 y	2.06 [a]	**0.0025**	1.27	0.41
Male gender	17%	43%	3.94	**0.0019**	1.93	0.16
Hypertension	29%	76%	7.98	**<0.0001**	4.65	**0.0098**
Diabetes	16%	38%	3.47	**0.0058**	0.955	0.93
OSA	13%	19%	1.56	0.42		
BMI	45	46	1.06 [b]	0.67		
NASH	10%	38%	5.28	**0.0002**	2.95	**0.027**
NASH with fibrosis	8%	33%	6.02	**0.0002**		

BMI, body mass index; HR, hazard ratio; OSA, obstructive sleep apnoea.
[a] HR for 10 year increase.
[b] HR for 5 kg/m^2 increase.

Conclusions: In a well characterised cohort of 493 morbidly obese patients undergoing bariatric surgery with a mean follow-up of more than 9 years, presence of NASH at liver biopsy and arterial hypertension were independently associated with overall mortality.

Note: LRB and EG contributed equally to this work.

P0991

RECIPIENT AND DONOR ADIPONECTIN POLYMORPHISMS ARE NOT ASSOCIATED WITH NEW ONSET DIABETES AFTER LIVER TRANSPLANTATION

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Background and Aims: Post-transplant metabolic syndrome is an important cause of morbidity and mortality in liver transplant recipients. Adiponectin is an adipokine with anti-inflammatory and anti-atherogeneic properties and hypoadiponectinemia may be associated with post transplant atherosclerosis, metabolic syndrome and insulin resistance. We have demonstrated that Adiponectin polymorphisms are associated with post-transplant hepatic steatosis but it is unclear if this effect is mediated by post transplant metabolic syndrome. In this study, we explored the association between donors and recipient Adiponectin genetic polymorphisms and New Onset Diabetes After Transplantation (NODAT).

Methods: We identified consecutive patients who underwent liver transplantation (LT) for HCV cirrhosis between 2006–2011 at a tertiary care center in the United States. This was a case-control study with cases and controls defined as subjects with and without NODAT respectively. New onset diabetes was defined as subjects with no evidence of diabetes prior to LT and documented

diabetes post-LT and was defined according to the WHO and American Diabetes Association 2003 criteria based on a fasting blood glucose level of greater than 126 mg/dl or the presence of diabetic symptoms and post prandial sugar of >200 mg/dl.
Donors and recipients were tested for the rs1501299, rs2241766, rs266729 and rs2241766 Adiponectin polymorphisms using the QLAamp DNA Mini Kit assay testing by TaqMan SNP genotyping assay.
Results: Clinical data were collected for a total of 302 patients who were transplanted for HCV during the study period. A total of 112 patients are included in the analysis. Mean age of recipient at time of transplant was 56±7 years and 73% were male. 21% developed NODAT (cases). Cases and controls were well matched in terms of age, gender, donor risk index, cold ischemia time, immunosuppression used, HCV genotypes as well as pre and post transplant metabolic syndrome. There was a trend toward association of NODAT in recipients without CMV (p = 0057), HCV genotype 1 (p = 0.087) and higher donor risk index (p = 0.051) but none were statistically significant. On both univariable and multivariable analysis, donor or recipient rs1501299, rs266729 and rs17300539 polymorphisms were not associated with NODAT.

Table: Association of genotypes and new onset diabetes: logistic regression analysis

Factor	Unadjusted		Adjusted [a]	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Recipient rs1501299: GG vs non-GG	1.4 (0.54, 3.4)	0.51	1.3 (0.52, 3.4)	0.56
Donor rs1501299: GG vs non-GG	1.2 (0.46, 2.9)	0.75	0.96 (0.38, 2.5)	0.94
Recipient rs2241766: TT vs non-TT	1.5 (0.45, 4.8)	0.53	1.3 (0.40, 4.1)	0.67
Donor rs2241766: TT vs non-TT	2.3 (0.64, 8.7)	0.2	2.3 (0.65, 8.0)	0.2
Recipient rs266726: CC vs non-CC	0.84 (0.32, 2.2)	0.73	0.93 (0.34, 2.5)	0.88
Donor rs266726: CC vs non-CC	0.87 (0.34, 2.2)	0.78	1.07 (0.40, 2.8)	0.9
Recipient rs17300539: GG vs non-GG	9.4 (0.49, 180.8)	0.14	7.4 (0.38, 141.0)	0.19
Donor rs17300539: GG vs non-GG	0.56 (0.18, 1.8)	0.32	0.55 (0.17, 1.8)	0.32

CI, confidence interval; OR, odds ratio.
[a] Adjusted for HCV genotype (1 vs. other).

Conclusions: Recipient and donor Adiponectin gene polymorphisms are not associated with NODAT. It appears that the association of Adiponectin polymorphisms and post transplant steatosis is independent of insulin resistance.

P0992
METABOLIC DISTURBANCES AFTER FRUCTOSE OVERCONSUMPTION ARE NOT LINKED TO INTESTINAL PERMEABILITY IN HEALTHY YOUNG VOLUNTEERS

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Background and Aims: Fructose overconsumption in Western diet is linked to non-alcoholic fatty liver disease (NAFLD). In animal models fructose overconsumption leads to chronic metabolic low-grade inflammation and was also linked to cardiovascular disease (CVD) as well as development of type 2 diabetes (T2DM). Until now, the impact of fructose overconsumption is often studied in overweight, T2DM or NAFLD patients, but is poorly understood in healthy subjects. To test our hypothesis that fructose overconsumption may impact on the gut–liver axis by increasing intestinal permeability, bacterial translocation, and consecutively metabolic inflammation as important mechanisms in the pathogenesis of NAFLD, CVD and T2DM, we examined the impact of a high oral fructose challenge (FC; 150 g/day for 4 weeks) on intestinal permeability and inflammation.
Methods: Ten healthy volunteers were enrolled (m:f = 5:5; median [range] age 24.5 [21–37] years; p = n.s.). Intestinal permeability was assessed by sucrose–lactulose–mannitol (SLM) test using high performance liquid chromatography of urine collected over 5 hours. CRP and fibronectin were assessed using nephelometry. Fetuin-A was measured using a commercial available ELISA Kit.
Results: After FC changes in energy homeostasis reflected by increased BMI (mean±SD 21.11±2.68 vs 21.51±2.77 kg/m^2; p < 0.001), fasting glucose levels (mean±SD 83.2±8.37 vs 88.4±5.48 mg/dL; p = 0.035) and increased fetuin-A levels during an oral glucose tolerance test at 90 min (260.9±30.1 vs 277.2±43.3 μg/ml; p = 0.046) and 120 min (234.8 [224.1–332.2] vs 288.1 [238.4–372.5] μg/mL; p = 0.007) were observed. Gastroduodenal permeability, reflected by recovered sucrose (p = 0.68) as well as small intestinal permeability reflected by lactulose/mannitol ratio (p = 0.622) remained stable. In line, inflammatory parameters CRP (p = 0.066) and fibronectin (p = 1.0) remained unchanged.
Conclusions: Fructose overconsumption (150 g/day for 4 weeks) in healthy young volunteers on top of their normal diet induces metabolic alterations without any increase in intestinal permeability or inflammation. This further supports the hypothesis that fructose overconsumption may act as "hepatotoxin" as a second hit in pre-existing metabolic diseases and/or on top of genetically predisposing factors such as PNPLA3 genotype.
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P0993
LOBULAR ENRICHMENT OF REGULATORY T CELLS WITHIN LIVERS OF PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: NAFLD is a metabolic disease with dramatically increasing relevance for the health care systems all over the world. The transition from steatosis to hepatitis with the subsequent risk of irreversible liver damage and carcinogenesis is incompletely understood. So far the innate and not the adaptive immune system is thought to be the immunological driver of liver inflammation.
Methods: We analyzed the intrahepatic T cell infiltration compartment specific (portal/lobular) employing a multicolor immunofluorescence in 32 liver biopsies of NAFLD patients.
Results: CD4$^+$FOXP3$^+$ regulatory T cells (Treg) significantly accumulated in the lobules, the focus of inflammation, and not in the portal fields with increasing histological disease activity (NAS-score). Thus NASH showed a significantly higher lobular Treg infiltration than NAFL, what was mirrored by increased Treg frequencies in the peripheral blood of NASH patients. Treg infiltration seemed to be more related to steatosis and hepatocyte ballooning than to lobular inflammation. Furthermore lobular CD4$^+$ Teff are significantly higher in NASH than NAFL and are correlated with the AST levels. In this cohort of mostly pre-obese patients we found no correlation of T cell infiltration with adiponectin, leptin and caspase cleaved cytokeratin-18 levels (M30).
Conclusions: In conclusion we found a compartment specific intrahepatic regulation of Treg with an accumulation at the site of inflammation according to the severity of NASH. The significant changes in the intrahepatic and peripheral T cell compartment point to a relevant involvement of the adaptive immune response in the pathogenesis of NASH.

P0994

CONTROLLED ATTENUATION PARAMETER (CAP) FOR DETECTION OF HEPATIC STEATOSIS IN PATIENTS WITH CHRONIC LIVER DISEASES

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Background and Aims: The objective of this study was to evaluate the performance of controlled attenuation parameter (CAP) to assess liver steatosis in patients with chronic liver diseases, and to analyse the relationships between CAP value and clinical or biological parameters in patients.
Methods: A total of 1152 consecutive patients with suspected chronic liver disease who completed CAP examination were enrolled. Of them, there were 121 patients who had undergone liver biopsy. Histological steatosis was categorized into S0 (<5%), S1 (5–33%), S2 (34–66%) and S3 (>66% of hepatocytes). The performance of CAP for evaluation of hepatic steatosis compared with liver biopsy was calculated by area under receiver operating characteristic curves (AUROC). In addition, the liver function and blood lipid level of the patients over the same period were detected. And the correlations of CAP with these data were analysed.
Results: The median age (875 males and 277 females) was 44 years old. The optimal cut-off value for CAP with steatosis suggested by abdominal ultrasound was 260 dB/m, and this study showed a good agreement of both methods (Kappa = 0.638, P < 0.001). In the 121 patients with liver biopsy, the median CAP values with S0, S1, S2 and S3 were 195 dB/m (range, 100–306), 236 dB/m (range, 146–336), 271 dB/m (range, 229–328) and 297 dB/m (range, 274–319) respectively. The cut-offs for the CAP value with steatosis ≥5%, ≥34% and ≥67% were 222 dB/m, 246 dB/m and 273 dB/m respectively. For the diagnosis of steatosis ≥5%, ≥34% and ≥67%, the areas under the curves were 0.88 (95% CI 0.82–0.95), 0.92 (95% CI 0.87–0.97) and 0.94 (95% CI 0.90–0.99), respectively. By multivariate analysis, factors associated with Controlled Attenuation Parameter (CAP) were BMI (P < 0.001), TG (P < 0.001), ALB (P < 0.001) and fasting glucose (P = 0.018).
Conclusions: CAP is a very good marker for the detection of hepatic steatosis. It was positively correlated with the level of serum lipid markers and impair of liver function.

P0995

THE USE OF THE ATTENUATION COEFFICIENT COMPUTED ON THE ULTRASONIC IMAGE COULD IMPROVE THE SPECIFICITY OF THE CLASSICAL ULTRASONOGRAPHIC EXAMINATION FOR THE ASSESSMENT OF STEATOSIS GRADE IN DIFFUSE LIVER DISEASES PATIENTS

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Background and Aims: Usual ultrasonography (US) is a useful diagnosis method in chronic liver disease (CLD) patients, but it cannot formulate an accurate quantification of fat content and cannot differentiate steatosis from fibrosis. We aim to evaluate the performance of a new parameter – attenuation coefficient (AC) computed on the US image in quantifying steatosis in CLD, compared to classical US examination.
Methods: 682 consecutive histological proven CLD patients (46.27±10.33 years, 57.2% females, 508 HCV, 74 HVB, 100 NASH) were prospectively included in this study. Liver fibrosis and necro-inflammatory activity were evaluated according to the Metavir scoring system in HCV and HBV, respectively Brunt in NASH. Steatosis was categorized as S0: <5%, S1: 6–33%, S2: 34–66% and S3: 67–100% of hepatocytes. They were referred to an US exam 1 day prior to liver biopsy (LB), using the same setting of a GE Logiq 7 device. Along a region of interest from easch US image there were stored the average gray level and the depth. Linear regression by least-squares approximation was applied to this dataset. The slope represents the attenuation coefficient (AC). Its diagnostic performance was compared with the performance of classical US examination.
Results: The univariate analysis provides a significant correlation between AC and steatosis (p < 0.001), but no correlation with fibrosis (r = −0.053, p = 0.214) and necroinflamatory activity (r = −0.054, p = 0.220) in HCV and HBV patients. In NASH, AC was significantly correlated with steatosis (r = −0.326, p = 0.001), ballooning (r = −0.234, p = 0.033) and lobular inflammation (r = −0.288, p = 0.009), with no significant correlation with fibrosis (r = −0.013, p = 0.897). In multivariate analysis, only steatosis was significantly correlated with AC (p < 0.001). The AC diagnostic performance in quantifying each steatosis grade was: for S ≥1 AUROC = 0.790 (cutoff = −0.0471, Se = 50.73%, Sp = 92.72%, PPV = 88.8%, NPV = 62.4%); for S ≥2, AUROC = 0.853 (cutoff = −0.0821, Se = 79.66%, Sp = 76.47%, PPV = 43.1%, NPV = 94.4%), respectively for S = 3, AUROC = 0.845 (cutoff = −0.1474, Se = 66.67%, Sp = 91.60%, PPV = 32%, NPV = 97.1%). AC increases the specificity of usual US from 84.84% to 92.72% for S ≥1 (p, 0.0001), from 70.39% to 76.47% for S ≥2 (p = 0.014), respectively from 87.85% to 91.60% for S3 detection (p = 0.03).
Conclusions: The use of the attenuation coefficient computed on the US image could substantially improve the ability of the usual US for the assessment of steatosis grade.

P0996

THE EFFECTS OF SHORT-TERM EXENATIDE ON CIRCULATING ADIPONECTIN LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS (DMT2) WITH OR WITHOUT NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD): A RANDOMIZED, OPEN-LABEL, CONTROLLED INTERVENTION TRIAL

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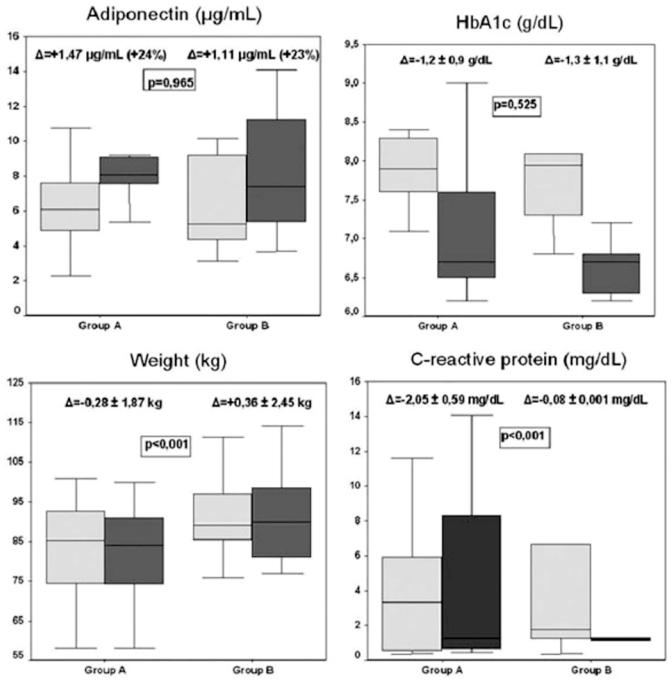
Background and Aims: DMT2 and NAFLD are both characterized by decreased circulating adiponectin levels. Furthermore, replenishment of hypoadiponectinemia is able to restore insulin sensitivity and to reverse weight gain, hyperglycemia and fatty liver. Recently, glucagon lipid peptide-1 receptor agonists like exenatide, have been shown not only to exert their action within liver parenchyma, but also to induce adiponectin expression. However, strong evidence of their interaction is still lacking.
Methods: DMT2 patients with abnormal aminotransferases were screened for NAFLD and subjected to liver biopsy to confirm/stage disease. A subgroup of patients, after assessed for eligibility criteria, was blindly randomized to receive either 6-month exenatide supplementation on glargine insulin (group A) or intense, self-regulated, insulin therapy alone (group B). Serum adiponectin was measured with ELISA before and after intervention, and several determinants like weight, BMI, waist circumference, aminotransferases, high-sensitivity C-reactive protein, HbA1c and lipidemic profile were recorded.
Results: From 470 DMT2 patients who had their aminotransferases for ≥6-month-period accessible in hospital databases, 110 took part

in the intervention trial for a mean duration of 6.7 months (finally analyzed group A n = 55 vs. group B n = 48). Baseline characteristics: 41 (39.8%) males, aged 62.9±7.2 years, weight 87±12.2 kg, BMI 31.2±5.5 kg/m^2, HbA1c 8.29±1.49%, median ALT 23 (5–126), AST 20 (7–72) U/L, 78 (15.6%) patients with abnormal ALT, 17 (3.6%) biopsy-proven NAFLD [median Activity Score 5 (2–7), fibrosis stage 3 (1–4)].

The graph shows the results of intervention. Adiponectin was significantly raised during intervention in both groups (Student's t for paired samples, $p < 0.001$) but this elevation was not attributable to the type of intervention, the degree of glycemic control, level of CRP or alterations in somatometrics.

Presence of NAFLD was accompanied by a significant decline in adiponectin ($p < 0.001$), which was found to negatively correlate with the degree of ALT in all groups (Spearman's correlation, $r_s = -0.544$, $p < 0.001$).

Conclusions: Supplementation of exenatide to glargine insulin compared to standard insulin was: (i) effective in inducing weight loss, (ii) non-inferior in lowering HbA1c and (iii) non-inferior in raising circulating adiponectin. Higher serum adiponectin concentrations were associated with lower ALT levels in all patient groups, confirming the hepatoprotective role of this cytokine.



P0997

WFA$^+$-M2BP IS A PREDICTIVE FACTOR FOR FIBROSIS PROGRESSION IN NAFLD

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Background and Aims: The *Wisteria floribunda* agglutinin-positive Mac-2 binding protein (WFA$^+$-M2BP) using the glycan "sugar chain"-based immunoassay was recently shown to be a liver fibrosis marker. Moreover, the feasibility of WFA$^+$-M2BP for assessing liver fibrosis progression in non-alcoholic fatty liver disease (NAFLD) was proven with clinical samples. On the other hand, cytokeratin-18 (CK-18) fragments levels are regarded as predictor of severity of NAFLD. The aim of this study was to compare the serum WFA$^+$-M2BP and CK-18 levels with clinical assessments of fibrosis and to compare WFA$^+$-M2BP with other fibrosis markers.

Methods: Serum WFA$^+$-M2BP and CK-18 levels were evaluated in 51 patients with NAFLD who had undergone liver biopsy. Histological findings were evaluated by three experienced, liver-specific pathologists. We analyzed predictive factor for fibrosis progression in NAFLD and compared WFA$^+$-M2BP with hyaluronic acid, type 4 collagen 7s, FIB-4 index, and NAFLD fibrosis score.

Results: The median age of the 51 patients (23 men and 28 women) was 59 (29–92) years old. Serum WFA$^+$-M2BP levels in patients with fibrosis stages 0/1/2/3/4 were 0.49±0.08/0.77±0.24/1.38±0.36/1.61±0.73/3.74±2.92 and there was significant difference between fibrosis stages 0–1 and 2–4 ($P < 0.001$). Multivariate analysis showed that FIB-4 index 2.0≤ ($P = 0.010$), WFA$^+$-M2BP 1.0≤ ($P = 0.020$), and albumin 4.0> ($P = 0.043$) were independently associated with the presence of fibrosis (Stage 2–4). CK-18 wasn't associated with the progression of fibrosis. The AUROC curve values for differentiating Stage 2–4 from Stage 0–1 were compatible for the serum WFA$^+$-M2BP (0.964), hyaluronic acid (0.926), type 4 collagen 7s (0.978), FIB-4 index (0.946), and NAFLD fibrosis score (0.917).

Conclusions: WFA$^+$-M2BP is one of the non-invasive predictors of fibrosis progression in NAFLD.

P0998

EVALUATION OF CIRCULATING PROGENITOR CELLS LEVELS AND FUNCTIONAL CAPACITY IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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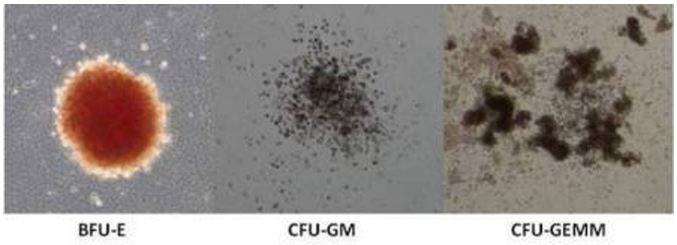
Background and Aims: The functions and the levels of progenitor cells are closely linked to metabolic syndrome. Thus, in this study we investigated the level and functions of circulating progenitor cells (CPC) in patients with NAFLD.

Methods: The study cohort consisted of 82 patients [37 with NAFLD (mean age 60 years, 75% male, 64% CAD) and 45 matched controls (mean age 64 years, 75% male, 68% CAD). Each patient had fasting serum, clinical data and abdominal ultrasound. NAFLD was defined as radiologic fatty liver in the absence of other causes of liver disease and excessive alcohol use. Circulating progenitor cells (CPC) were quantified by flow cytometry based on the expression of (CD34$^+$, CD133$^+$, CD34$^+$ CD133$^+$) in presence or absence of the hematopoietic marker (CD45). To study the functional capacity of CPC, colony-forming unit (CFU) assay was used. These include erythroid progenitors [burst-forming unit erythroid (BFU-E)]; granulocyte/macrophage progenitors [CFU-granulocyte, macrophage (CFU-GM)]; and multi-potential progenitors [CFU-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM)].



Results: The level of CD45$^+$CD133$^+$ cells was higher in NAFLD patients (Median, 0.19%) than in controls (median, 0.16, $p = 0.003$). NAFLD patients in the lowest quartile (25th) of CD45$^+$CD133$^+$ cells had the lowest risk for NAFLD [OR: 0.29 (95% CI: 0.10–0.84)]. In contrast CFU-GM [OR: 5.92 (95% CI: 1.51–23.24)] and CFU-GEMM [OR: 5.18 (95% CI: 1.31–20.57)] were associated with increased risk

for NAFLD for the same quartile. In multivariate analysis, presence of $CD45^+CD133^+$ cells was independently protective [OR: 0.29 (95% CI: 0.08–0.99)] against NAFLD.
Conclusions: NAFLD patients have an increased level of circulating progenitor cells $CD45^+CD133^+$, possibly due to impaired differentiation capacity of these progenitors to the committed cell line. Furthermore, functional CPS may play a protective role against development of NAFLD.

P0999
SERUM VITAMIN D DEFICIENCY IN NON-ALCOHOLIC FATTY LIVER DISEASE; CHICKEN OR THE EGG?

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Background and Aims: Serum Vitamin D concentrations are reduced in non-alcoholic fatty liver patients (NAFLD); however, there is no data whether it is caused by reduced dietary intake, reduced sunlight exposure, or liver disease itself [reduced 25(OH)D conversion]. We would like to check the change of serum vitamin D level after calorie intake restriction without changing sunlight exposure in non-alcoholic fatty liver disease (NAFLD) patients.
Methods: Ninety sonographic fatty liver disease subjects were enrolled. All participants received nutritional education for two months. Dietary intakes were assessed using three-day food records. Dietary education program was served to take 25kcal/kg according to ideal body weight to reduce weight. Sunlight exposure and physical activity were also assessed.
Results: Eighty-two participants finished 8 weeks education program. At baseline, the average serum vitamin D concentrations were 13.0 ng /mL. Serum vitamin D level was lower than 20 ng /mL in 74 patients (91.1%), and 29 patients (35.4%) had less than 10 mg/mL. Although vitamin D intake (4.9 μg/day vs. 4.0 μg/day, $p = 0.14$) as well as total calorie uptake were decreased, serum vitamin D concentration increased (13.0 ng/mL vs. 15.9 ng/mL, $p < 0.001$). Increasing of serum vitamin D level was associated with body weight reduction not by vitamin D intake, and sunlight exposure. The participants had the same intensity of physical activities and sunlight exposure during the study period as before. Serum vitamin D level was independent risk factor for normalization of aminotransferase activities and improvement hepatic fat contents as assessed with liver HU and liver spleen HU ratio.
Conclusions: Weight loss induced improved fatty liver increases serum vitamin D concentration independent of vitamin D intake and sunlight exposure.

P1000
PREVALENCE AND PREDICTORS OF TRANSIENT ELASTOGRAPHY-DEFINED NON-ALCOHOLIC FATTY LIVER DISEASE WITH AND WITHOUT SIGNIFICANT LIVER FIBROSIS AMONG ASYMPTOMATIC SUBJECTS UNDERGOING A MEDICAL HEALTH CHECK-UP

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Background and Aims: Transient elastography (TE) can accurately assess the degree of liver fibrosis and steatosis. However, few data have been available regarding the clinical role of TE in screening general population and identifying high-risk sub-population. Thus, we have investigated the prevalence and predictors of non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) among asymptomatic subjects undergoing a medical health check-up.
Methods: Between April 2013 and August 2014, a total of 2,944 subjects without alcoholic and chronic viral liver diseases who received a medical health check-up including TE examination were consecutively recruited. NAFLD was defined as Controlled Attenuation Parameter (CAP) >250 dB/m, significant fibrosis as liver stiffness (LS) >7 kPa, and non-alcoholic steatohepatitis (NASH) as both CAP >250 dB/m and LS >7 kPa.
Results: The mean age of the population in this study (men 1,761 and women 1,183) was 54 years. Among them, 121 (4.1%) subjects had significant fibrosis, and 1,290 (43.8%) had NAFLD. Subjects with NAFLD were significantly older and had higher alanine aminotransferase (ALT) level, higher prevalence of parameters related to metabolic syndrome such as high blood pressure, high body mass index (BMI), glucose intolerance, and dyslipidemia than those without NAFLD (all $P < 0.05$). Independent predictors of NAFLD were age, BMI, ultrasonographic fatty liver, the amount of visceral fat, serum albumin, hypertension, diabetes, and high-density lipoprotein (all $P < 0.05$). In addition, NASH was identified in 84 (2.9%) subjects. Its independent predictors were male gender (odds ratio [OR] 3.435, 95% confidence interval [CI] 1.328–8.885, $P = 0.011$), age (OR 1.041, 95% CI 1.003–1.081, $P = 0.032$), high BMI (OR 1.285, 95% CI 1.071–1.542, $P = 0.007$), hypertension (OR 3.004, 95% CI, 1.731–5.213, $P < 0.001$), and diabetes (OR 2.993, 95% CI 1.525–5.873, $P = 0.001$).
Conclusions: Using TE, we identified the prevalence of NAFLD (43.8%) and NASH (2.9%) and their predictors in asymptomatic subjects undergoing a medical healthy check-up. Specifically, subjects with risk factors of NASH such male gender, high BMI, hypertension, and diabetes should be screened using TE for early identification of disease progression in spite of no history of alcoholic or chronic viral hepatitis.

P1001
CAROTID-FEMORAL PULSE WAVE VELOCITY (CF-PWV) AS A SURROGATE MARKER OF ADVANCED LIVER FIBROSIS IN TYPE-2 DIABETIC (T2DM) PATIENTS EVALUATED BY TRANSIENT HEPATIC ELASTOGRAPHY

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Background and Aims: So far, it is not known whether there is any association between cf-PWV, the gold standard measure of aortic stiffness, and advanced liver disease. We aimed to evaluate the association between cf-PWV among diabetes and non-diabetes-related factors and advanced fibrosis identified by transient hepatic elastography (THE) (Fibroscan®, Echosens, France) in T2DM patients.
Methods: Baseline and follow-up clinical, laboratory and cf-PWV data from T2DM outpatients were obtained. A second cf-PWV was registered. The cutoff value for considering increased cf-PWV was 10 m/s. Liver fibrosis was evaluated by THE with controlled attenuated parameter (CAP) on a follow up time of 7 years after baseline. At THE, a cutoff value above 7.9 kPa (M probe) and 7.2 kPa (XL probe) were considered as advanced fibrosis. Data was retrospectively analyzed and the independent factors associated to advanced fibrosis were identified by logistic binary regression. A significance level of 0.05 was adopted.
Results: Two hundred and twenty-one T2DM patients [male 74 (34%), mean age 58±8 yrs] with a BMI of 30±5 kg/m^2 and mean weight of 77±13 kg were included. Only 89 (40%) patients had glycated hemoglobin less than 7%. A cf-PWV >10 m/s was observed in 16% and 28% in the first and second measurement,

respectively and 132 patients (60%) increased their initial cf-PWV values. Advanced fibrosis was identified in 69 (31%) patients and liver steatosis above 66% was observed in 42% of patients. Surprisingly, there was no association between advanced fibrosis and T2DM parameters (diabetes duration, macro and microvascular complications prevalence at baseline) neither with diabetes-related drugs. In two different models of multivariate logistic regression, both a second PWV >10 m/s (OR: 2.46, 95% CI: 1.14–5.32; p = 0.021) and an evolutive increase in PWV of at least 2 m/s (OR: 2.65, 95% CI: 1.08–6.49; p = 0.03) were independently associated with greater odds of having advanced liver fibrosis as well as persistently high GGT levels (OR: 5.76, 95% CI: 2.16–15.38; p < 0.01), HDL-cholesterol levels (reduction of 1 mmol/L, OR: 1.03, 95% CI: 1.06–2.0; p = 0.04) and increased steatosis diagnosed by the CAP (increment of 1 dB/m, OR: 1.01; 95% CI: 1.00–1.02; p < 0.01).

Conclusions: Cf-PWV measurement is a non-invasive tool that may be a useful surrogate marker of advanced liver fibrosis in T2DM patients. These findings suggest that it might also be valid in patients with NAFLD, which is the main cause of chronic liver disease in diabetic patients.

P1002

THE HISTOLOGICAL SEVERITY OF NAFLD DETERMINES THE CARDIOVASCULAR RISK AND INSULIN RESISTANCE IN METABOLICALLY HEALTHY AND UNHEALTHY OVERWEIGHT INDIVIDUALS

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Background and Aims: Obesity is a heterogeneous condition in terms of metabolic and cardiovascular (CV) risks, which are attributed to the presence of metabolic risk factors (MRF) rather than obesity *per se*. The prevalence and severity of NAFLD in different categories of obese patients (pts) and its impact on insulin resistance (IR) and CV risk could, however, confound this relationship.

Aim: To explore the prevalence, determinants and impact of the histological severity of NAFLD on IR and 10-year CV risk in a heterogeneous population of overweight pts.

Methods: 401 pts with biopsy-proven NAFLD (Hepatology Cohort-HC) and 626 morbidly obese pts (Bariatric Cohort-BC). NAFLD/NASH was defined according to SAF classification; significant fibrosis: ≥F2. Metabolically healthy (MH) individuals were defined as non-diabetic pts with ≤1 of the following MRF: visceral obesity, arterial hypertension, hypoHDL. Pts were classified into 3 categories based on the above MH status and overweight (BMI≥25 kg/m^2): metabolically healthy and unhealthy overweight (MHO, MUHO) and normal weight (NW) pts with/without MRF. The Framingham Score (FRS) was calculated.

Results: HC: 62% of patients were males; mean age 54±12 years; mean BMI 30.7±6.1 kg/m^2; 70% NASH; 49% ≥F2; mean FRS 11±8%. 30% of patients were MHO, 55% MUHO and 15% NW. The prevalence and severity of histological lesions gradually increased across groups (Table).

These results were reproduced in 200 age and sex-matched pts from BC (2:1 random selection).

The distribution of HOMA and FRS across groups (NW, MHO, MHNO) was: 3.9±4.4, 4±4.1, 6.2±6.1, p = 0.001 and 11±8, 8±7, 13±8, p < 0.001, respectively. Pts with advanced NASH (NASHF2) vs. those without, had higher FRS in both MHO (10±8 vs. 7±6 p = 0.015) and MUHO (15±8 vs. 11±8, p = 0.003), but not in NW pts. In the entire cohort, NASHF2 predicted IR (beta=0.145, p = 0.008) and FRS (beta=0.081, p = 0.036) independent of MRF and body weight, in a model adjusted for age and sex.

	NW	MHO	MUHO	P, MHO vs.	
				MUHO	NW
NASH	49	68	75	0.14	0.016
Steatosis grade 3 or 4	15	16	26	0.05	0.76
Activity Score >2	18	36	51	0.006	0.012
≥F2	30	41	58	0.003	0.12

Conclusions: In pts with excessive body weight advanced NAFLD (NASH with fibrosis) independently determines CV risk and IR above and beyond MRF and the degree of overweight. In turn, MRF confers a higher risk of histologically severe NAFLD regardless of body weight. The NAFLD phenotype needs to be assessed in overweight pts to better characterize their overall health risks.

P1003

NONINVASIVE PREDICTION OF EROSIVE ESOPHAGITIS USING A CONTROLLED ATTENUATION PARAMETER (CAP)-BASED RISK ESTIMATION MODEL

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Background and Aims: Erosive esophagitis and fatty liver share obesity and visceral fat as common critical pathogenesis. We evaluated the relationship between the amount of hepatic fat measured by controlled attenuation parameter (CAP) using transient elastography and the presence of erosive esophagitis, and then developed a CAP-based risk estimation model for erosive esophagitis.

Methods: We enrolled 1,045 consecutive participants (training cohort, n = 705; validation cohort, n = 340) from a health check-up center who underwent esophagoduodenoscopy and CAP. Independent predictors for erosive esophagitis that have been investigated through logistic regression analyses were used as components for establishing a risk estimation model.

Results: Among the entire population, the prevalence of erosive gastritis was 10.7% (9.4% in the training cohort; 13.5% in the validation cohort). A CAP-based risk estimation model for erosive esophagitis using CAP, Body mass index, and significant alcohol Drinking as constituent variables was established, and was dubbed the CBD score. The CBD score ranged from 0 to 11, with the accuracy showing an area under the receiver operating characteristic curve (AUROC) of 0.819 for predicting erosive esophagitis. The high-risk group (CBD score ≥3) showed significantly higher risk of having erosive esophagitis than the low-risk group (CBD score <3) (24.1% vs. 2.7%, respectively; P < 0.001). The diagnostic accuracy of CBD score was maintained in the validation cohort (AUROC = 0.848).

Conclusions: The CBD score is a simple CAP-based risk model for predicting erosive esophagitis. The CBD score may be the alternative option for EGD in monitoring the occurrence of erosive esophagitis, considering its convenience and non-invasiveness.

P1004
IMPACT OF AEROBIC EXERCISE IN POSTMENOPAUSAL WOMEN WITH NONALCOHOLIC FATTY LIVER DISEASE: A 24 WEEKS RANDOMIZED CLINICAL TRIAL

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) is common in postmenopausal women and it is associated with an increased prevalence of cardiovascular disease. The aim of this study was to establish the effectiveness of aerobic exercise and its influence in reducing metabolic and cardiovascular risk in postmenopausal women with NAFLD.
Methods: Forty sedentary postmenopausal women (mean age 55.3±8.0 years), range BMI (24.2–43.3 kg/m^2) biopsy-proven NAFLD were randomly divided into two parallel groups: Exercise Group (19 patients) and Control Group (21 patients). The exercise group performed a supervised aerobic exercise program of 60min/2 days/week at 60–85% of heart rate reserve for 24 weeks. The anthropometric parameters, body composition, hepatic, lipid, and glycemic profiles, HOMA-IR, cytokines (IL8, TNF and VEGF), transient elastographyby FibroScan (FS) – CAP and cardiorespiratory exercise test on the treadmill (Centurion 200, Micromed) were evaluated at baseline (T0) and after 24 weeks of protocol (T1). All patients were advised to use hypocaloric diet, with monthly returns with nutritionist.
Results: At baseline there were no statistically significant differencein the age, blood pressure, anthropometric parameters, percent body fat (PBF), hepatic, lipid, glycemic profiles, HOMA-IR, cytokines and liver histology/FS [liver stiffness (LS)/CAP] among the groups. We observed decrease of abdominal circumference (CA) ($p = 0.05$), BMI, and increase of HDL levels ($p < 0.05$) in the exercise group after 24 weeks. There were no statistically significant differences in the cytokines (IL8, TNF, and VEGF) between the groups. Regarding FS there was a stabilization of LS/CAP in the exercise group and a trend of increase of these parameters in the control group. The CAP analysis showed improvement of hepatic fat content in exercise group.
Conclusions: An aerobic exercise program of 24 weeks in NAFLD postmenopausal women showed improvement in some variables such as CA, BMI, HDL and hepatic fat content that may be beneficial in improving cardiovascular risk factors.

P1005
IS ASPIRIN USE A PROTECTOR FACTOR OF NONALCOHOLIC FATTY LIVER DISEASE?

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Background and Aims: Nonalcoholic fatty liver disease is now conceptualised as a 'multiple hit' process, consisting of insulin resistance, which causes a reversible fat accumulation in hepatocytes as the first hit, and a combination of oxidative stress, lipid peroxidation and pro-inflammatory cytokines as the subsequent hits. Aspirin may affect oxidative stress, vascular inflammation and insulin sensitivity.
Aim: To investigate an association between aspirin use and NAFLD prevalence in the patient with arterial hypertension.
Methods: A total of 625 patients (mean age: 48.15±10.5 years, 72% males) who had arterial hypertension and underwent ultrasonography were included in the study; of those, 167 patients (mean age: 49.62±9.97 years, age range: 23–73, 85/167 [50.9%] females) were identified as having NAFLD and 458 as control. Aspirin use during the month prior to interview was categorised as never use (0 times), occasional use (1–14 times) and regular use (≥15 times).
Results: Patient with NAFLD were more likely to be obese, physically inactive, have diabetes. This group were less frequently use aspirin, than patient from control group. In the multivariate unconditional logistic regression analysis, regular relative to no aspirin use was inversely associated with prevalent NAFLD. Odds ratio 0.23, 95% confidence interval (CI) 0.12–0.42; $p < 0.01$.
Conclusions: Regular aspirin use (≥15 times per month) associated with a lower prevalence of NAFLD in case of patient with arterial hypertention and may be had a protective effect in development of nonalcoholic fatty liver disease.

P1006
LIVER IRON CONCENTRATION IN THE METABOLIC SYNDROME WITH HYPERFERRITINEMIA (DYSMETABOLIC HYPERFERRITINEMIA). RESULTS FROM A COHORT OF PATIENTS STUDIED BY MRI

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Background and Aims: Nearly 25% of adult population in western countries have the metabolic syndrome (MS). Hyperferritinemia (HF) is frequently present in patients with this clinical entity (dysmetabolic hyperferritinemia). There are some publications that support that HF is associated with a raised liver iron concentration (LIC) in these patients, but some doubts persist about this subject.
To study the LIC in patients referred for hyperferritinemia to a secondary hospital in the Basque Country, Spain, and determine if there are differences between patients with or without metabolic syndrome.
Methods: A prospective study of 132 consecutive patients with HF (>200 μg/L women, >300 μg/L men) was conducted from January to December 2010. The Metabolic syndrome was defined by the presence of waist circumference ≥94 cm men/≥80 cm women and two of the following factors: Triglycerides ≥≥150 mg/dL or treatment for this dislipidemia; HDL <40 mg/dL women/<50 mg/dL men or treatment for this dislipidemia; glucose ≥100 mg/dL or Type 2 diabetes; hypertension: blood pressure ≥130 mmHg/≥85 mmHg or treatment for arterial hypertension. LIC was determined by MRI (SIR method).
Results: In 97 of 132 patients we have all the data to determine the MS presence: 44/80 men (55%) and 10/17 women (59%) presented MS. In 54/97 patients MRI for LIC determination (mean±SD) was performed. From the MS group (54 patients), 44 were men (27 had MRI) and 10 women (9 MRI). So that, we have LIC results (μmol/g) from 36 patients. The mean LIC was 27.63±20.18 (men) and 28.44±18.31 (women), with *27.83±20.90* for all the MS group. In 43/97 patients MS was not diagnosed (NMS): 36 were men (13 MRI), and 7 women (5 MRI). In 18 patients from the NMS group LIC by MRI was determined. The mean LIC was 30.84±23.79 in men, and 39.2±23.79 in women, with *33.16±19.61* for all the NMS group. We compare the mean values of LIC from both groups (MS vs NMS) by Pearson's Chi square test. No significant differences were retrieved ($p = 0.067$).
Conclusions: Patients with HF and MS (dysmetabolic hyperferritinemia) present a mean LIC within normal values and their values do not differ from those of patients with HF and without MS.

P1007

H63D/H63D GENOTYPE AND H63D ALLELE PREDISPOSE PATIENTS WITH HYPERFERRITINEMIA TO DEVELOP METABOLIC SYNDROME

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Background and Aims: Nearly 25% of adult population in western countries have the metabolic syndrome (MS). Hyperferritinemia (HF) is frequently present in patients with this clinical entity (dysmetabolic hyperferritinemia). The presence of mutations in the HFE gene, like the H63D/H63D mutation, may induce MS in these patients.
Methods: A prospective study of 132 consecutive patients with HF (>200 μg/L women, >300 μg/L men) was conducted from January to December 2010. The Metabolic syndrome was defined by the presence of waist circumference – ≥94 cm men, ≥80 cm women, and two of the following factors: triglycerides ≥150 mg/dL or treatment for this dislipidemia; HDL <40 mg/dL women, <50 mg/dL men or treatment for this dislipidemia; glucose ≥100 mg/dL or type 2 diabetes; hypertension: blood pressure ≥130 mmHg/≥85 mmHg or treatment for arterial hypertension. DNA was extracted from the blood samples and *HFE* gene analysis was performed by multiplex real-time PCR using LightCycler technology (LC 1.0). Simultaneous detection of the *HFE* C282Y, H63D and S65C mutations was carried out in a single capillary using LC-Red 640, LC-Red 705 and fluorescein-labelled hybridization probes (Tibmolbiol, Berlin, Germany). Melting curve analysis was used to distinguish wild type and mutant alleles in each case. The results were compared with a control group of blood donors from the same geographical area.
Results: In 97 of 132 patients we have all the data to determine the MS presence: 44/80 men (55%) and 10/17 women (59%) presented MS. The HFE mutations from 51/54 patients with MS were: 2 C282Y/wt (3.92%); 11 H63D/H63D (21.56%); 15 H63D/wt (29.41%); 3 C282Y/H63D (5.88%); 19 wt/wt (37.25%); 1 S65C/ wt (1.96%). The genotype frequency of the H63D/H63D mutation in MS cases was significantly higher than in controls (7) – 21.56% vs 7.76% ($p = 0.011$); the H63D allelic frequency was 42.15% in the MS group and 31% in controls ($p = 0.027$).
Conclusions: The H63D/H63D genotype and H63D allele predispose individuals with HF to MS.

P1008

VALUE OF CONTROLLED ATTENUATION PARAMETER (CAP) IN THE CLINICAL PRACTICE: PRELIMINARY RESULTS

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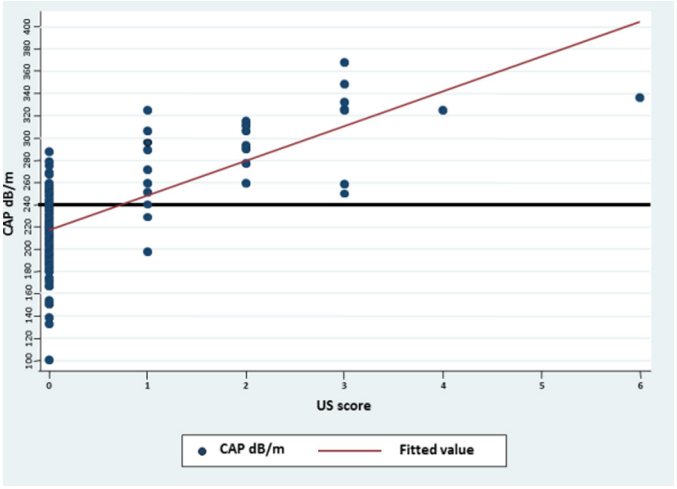
Background and Aims: Several studies have shown that controlled attenuation parameter (CAP), a new technique that noninvasively estimates the fat in the liver, is accurate in detecting liver steatosis. The aim of this study was to assess the role of CAP in the clinical practice.
Methods: Consecutive patients referred to the Ultrasound Unit of the Infectious Diseases Dept. of our Institution for abdominal ultrasound (US) examination were enrolled. Liver steatosis was assessed with US by using the Hamagouchi score, which is a 6-point scale. CAP was performed in all subjects by using the M+ probe of the FibroScan device (Echosens, Paris, France). For the detection of liver steatosis, US and CAP score greater than 0 and greater than 240 dB/m, respectively, were considered. A 2×2 contingency table and the Students *t* test were used to compare the group differences. Spearman rank coefficient was used to test correlation between CAP and US. Data were expressed as means and standard deviations.
Results: One hundred and thirty-three subjects [74 males and 59 females; mean age, 51.2 (SD: 13.9) yrs; BMI, 24.4 (6.5) kg/m^2] were studied. CAP measurements were not obtained in 6/133 (4.5%) of subjects due to skin to liver capsule distance >25 mm, thus the data of 127 subjects were analyzed. CAP and US were highly correlated ($r = 0.61$, $p < 0.0001$). US detected liver steatosis in 26/127 (20.5%) subjects; they showed a significantly higher CAP value with respect to subjects without US liver steatosis [291.7 (40.5) vs 214.4 (33.3) dB/m, $p < 0.0001$]. CAP was >240 dB/m in 22/101 (21.8%) subjects in whom liver steatosis was not detected at US. CAP was ≤240 dB/m in three subjects with liver steatosis assessed with US; these subjects showed liver stiffness values of 7.6, 8.7 and 11.5 kPa. In detecting liver steatosis, US had 51.1% sensitivity and 96.3% specificity.
Conclusions: These preliminary results show that CAP is more sensitive than US in the detection of liver steatosis. Liver fibrosis could be a confounding factor in the US detection of liver steatosis.



P1009

INCIDENCE OF MAJOR CARDIOVASCULAR AND CEREBRAL EVENTS IN PATIENTS WITH NAFLD AND IN CONTROLS OF GENERAL POPULATION DURING 10 YEARS OF FOLLOW UP: CORRELATION BETWEEN VASCULAR AND LIVER DAMAGE

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Background and Aims: NAFLD represents a risk factor for vascular damage. Carotid intima-media thickness (cIMT) is a known precursor of cardiovascular disease. Aims: to evaluate (1) risk factors affecting the progression of cIMT and early carotid plaques (CP) in patients with NAFLD and in a control group from general population, (2) incidence of major cardiovascular and cerebral events in ten years of follow up, (3) correlation between vascular and liver damage.

Methods: 125 patients with NAFLD diagnosed by ultrasonography matched 1:2 for sex and age with subjects from general population underwent vascular evaluation in 2003 and were prospectively followed for a period of 10 years. In all subjects cIMT by echo color doppler, clinical and biochemical data were evaluated at enrollment (time 0). After 10 years follow-up (time 1), 90/125 patients with NAFLD and 182/250 controls underwent abdominal ultrasonography to evaluate the presence of liver steatosis and a second cIMT measurements and CP evaluation, the remaining patients were lost at follow up. All clinical, biochemical data were recorded at time 0 and time 1.
Results: At enrollment cIMT was significantly more elevated in NAFLD than in controls (0.87±0.23 vs 0.64±0.14, p = 0.001) and the prevalence of CP significantly higher (21% vs 6%, p = 0.001). After 10 years 54/182 (30%) controls developed steatosis. cIMT remained significantly more elevated in NAFLD than in controls who developed steatosis (0.95±0.21 and 0.8±0.13 mm, p = 0.004), while the higher prevalence of plaques was observed in controls who developed steatosis (50%, 46% and 60%, in NAFLD, controls without and controls with steatosis respectively). Thirty-five subjects developed major cardiovascular and cerebral events, the prevalence was significantly higher in NAFLD and in controls with steatosis (p = 0.02). At logistic regression analysis variables significantly associated with events were age unit (OR 1.01, 95% CI 1.02–1.1, p = 0.004), systolic pressure (OR 1.05, 95% CI 1.01–1.08, p = 0.004), the presence of plaques (OR 3.78, 95% CI 1.5–8.8, p = 0.002) and basal ALT >35 (OR 3.7, 95% CI 1.08–14, p = 0.03).
Conclusions: Subjects of general population are at high risk of developing steatosis throughout their life, major cardiovascular events have the same prevalence in NAFLD and controls with the higher prevalence in controls who developed steatosis and are related with liver damage. All subjects with steatosis have to be considered at high risk for cardiovascular complications.

P1010
INFLAMMATION, OXIDATION AND ECTOPIC FAT ACCUMULATION IN MORBID OBESITY

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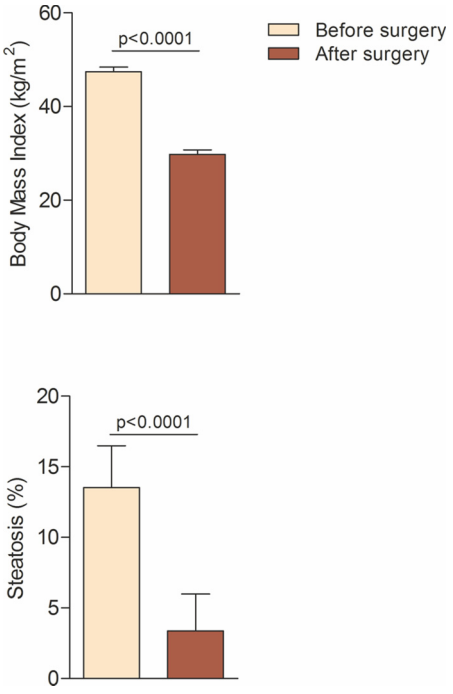
Background and Aims: Obesity severely affects human health. Non-alcoholic fatty liver disease (NAFLD) is associated with high morbidity and mortality and is characterized by the presence of increased amounts of fat in the liver (steatosis). The deleterious effects of the excessive energy intake are associated with inflammation and oxidation. Chemokine (C-C motif) ligand 2 (CCL2) is known to play a critical role in the migration of inflammatory cells and paroxonase-1 (PON1) and 4-hydroxynonenal (4-HNE) are related in lipid peroxidation and oxidative stress. The aim of this study was to better understand the pathogenesis of NAFLD in morbid obesity.
Methods: Patients undergoing bariatric surgery following procedures involving laparoscopic sleeve gastrectomy were recruited as a model of obesity-induced NAFLD in an observational, prospective, single-site, and cross-sectional study with a pre-set duration of 1 year. Samples were obtained in these patients immediately before undergoing bariatric surgery and after a 12-month period. Relevant data like BMI and histological features in the liver were obtained prospectively and surrogate markers of inflammation and lipid peroxidation (CCL2, PON1 and 4-HNE) were analysed in the liver by immunochemical staining analysis.
Results: We found a significant decrease in steatosis after surgery and also BMI was considerably reduced. In addition, the presence of CCL2, PON1 and 4-HNE was significantly increase in liver obtained before bariatric surgery compared to samples obtained after a 12-month period.
Conclusions: Bariatric surgery seems to reverse the progression of NAFLD since BMI, steatosis and the presence of inflammation and lipid peroxidation markers decrease in the liver of morbid obese patients after 12-month period of the surgery.



P1011
FIBROSCAN IS A USEFUL CLINICAL TOOL TO EXCLUDE SIGNIFICANT STEATOSIS IN CHRONIC LIVER DISEASE PATIENTS

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Background and Aims: Steatosis is a common histological finding in patient with chronic liver disease (CLD). Its detection is relevant because it may progress to fibrosis, cirrhosis, and hepatocellular carcinoma A novel non-invasive tool for steatosis assessment, called controlled attenuation parameter (CAP), is based on the evaluation of ultrasound attenuation using transient elastography (TE). We aim to establish utility of CAP in predicting each steatosis grade.
Methods: 225 CLD patients (48 with HBV, 130 with HCV, 47 with NASH), mean age 52 years, 63% females, were enrolled. All of them underwent percutaneous liver biopsy (LB) for grading and staging the disease and were referred for CAP measurement, 1 day prior to LB. In all patients, steatosis was categorized by visual assessment as S0: steatosis in less than 10% of hepatocytes, S1: 11%>33%, S2: 34%>66% and S3: 67%>100% of hepatocytes. The median size of the LB specimens was 15 (12–20) mm, with a median of 14 (10–22) portal spaces. The diagnostic performance of CAP for steatosis prediction was assessed using sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive value (NPV), positive (+LR) and negative (−LR) likelihood ratios and area under ROC curves (AUC).
Results: The median (range) CAP values (dB/m) according to the steatosis grades were: 212 (124–359) for S0; 266 (153–353) for S1; 304 (215–359) for S2 and 321 (218–377) for S3. The differences were statistically significant between

all the steatosis grades, except S2 vs S3. The diagnostic performances of CAP in quantifying each steatosis grade was: for S ≥1 AUC = 0.813 (cutoff 260 dB/m, Se = 64.84%, Sp = 87.27%, PPV = 80.8%, NPV = 75%, +LR = 5.09, −LR = 0.40, DA = 76.11%); for S ≥2 AUC = 0.822 (cutoff 285 dB/m, Se = 69.70%, Sp = 85.12%, PPV = 47.9%, NPV = 93.5%, +LR = 4.68, −LR = 0.36, DA = 82.08%); for S ≥3 AUC = 0.838 (cutoff 294 dB/m, Se = 83.33%, Sp = 82.54%, PPV = 23.3%, NPV = 98.7%, +LR = 4.77, −LR = 0.20, DA = 81.59%). AUCs calculated between two steatosis grades only were: 0.772 (for S0 vs S1), 0.874 (S0 vs S2), 0.904 (S0 vs S3), 0.659 (S1 vs S2), 0.777 (S1 vs S3), and 0.665 (S2 vs S3) respectively.
Conclusions: Maximal diagnostic accuracy could be obtained for the prediction of ≥S2 and S3 (82.06% and 81.59% respectively) while, for the prediction of ≥S1, the accuracy reached only 76.11%. CAP has a high negative predictive value (93.5% and 98.7% respectively) for ≥S2 and S3, which suggests that CAP could be an useful clinically tool to exclude these steatosis grades.

P1012
PROSPECTIVE COMPARISON OF NONINVASIVE FIBROSIS ASSESSMENT TO PREDICT ADVANCED FIBROSIS OR CIRRHOSIS IN ASIAN PATIENTS WITH NAFLD

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is one of the major causes of liver disease worldwide. Its prevalence continues to rise, and it threatens to become a serious health problem. This study aimed to evaluate the diagnostic accuracy of noninvasive fibrosis assessment to predict advanced fibrosis or cirrhosis in Asian patients with NAFLD.
Methods: One hundred sixteen patients with a liver biopsy-confirmed diagnosis of NAFLD were prospectively evaluated between March 2013 and September 2014. Liver stiffness measurement (LSM) was performed by acoustic radiation force impulse (ARFI) elastography in all patients. Aspartate aminotransferase to alanine aminotransferase ratio (AAR), Fib-4, aspartate aminotransferase to platelet ratio index (APRI), NAFLD fibrosis score and BARD scores were calculated according to published algorithms. Diagnostic measurements of serum fibrosis indices and ARFI imaging were compared to predict advanced fibrosis or cirrhosis by analyzing the area under the receiver operating characteristic (AUROC) curve.
Results: The median age of the study population was 54.3 years (range, 18–78). Fib-4, NAFLD fibrosis score, BARD score and LSM showed significant, positive correlations with METAVIR stages (P < 0.001). LSM by ARFI had the greatest AUROC for predicting advanced fibrosis (≥F3) (0.883; 95% CI, 0.804–0.961) and cirrhosis (F4) (0.926; 95% CI, 0.848–1.000). And Fib-4 had the good AUROC for predicting cirrhosis (F4) (0.873; 95% CI, 0.803–0.942). A tendency toward increasing liver stiffness existed in a graded fashion across METAVIR stages (P < 0.001).
Conclusions: Liver stiffness by ARFI was useful noninvasive fibrosis assessment for predicting advanced fibrosis and cirrhosis in Asian patients with NAFLD. In addition, A Fib-4 exhibited acceptable diagnostic performance in the assessment of hepatic fibrosis in patients with NAFLD.

P1013
CHRONIC INTERMITTENT HYPOXIA IS ASSOCIATED WITH LIVER DAMAGE AND ATHEROSCLEROSIS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Obstructive sleep apnea syndrome (OSAS) has been reported as a new risk factor for metabolic disturbances, including clinical and subclinical cardiovascular alterations. Growing data are also available among bariatric nonalcoholic fatty liver disease (NAFLD) patients on the impact of obstructive sleep apnea syndrome OSAS on liver damage in NAFLD. We assessed whether OSAS is associated with severity of liver fibrosis and carotid atherosclerosis in NAFLD patients without morbid obesity.
Methods: 126 consecutive biopsy-proven (Kleiner score) NAFLD patients assessed for anthropometric, biochemical, and metabolic features underwent ultrasonographic carotid assessment and STOP BANG questionnaire for estimate of low or high OSAS risk. A carotid plaque was defined as a focal thickening >1.3 mm at the level of either common and internal carotid arteries or bifurcations. 50 patients accepted to perform nocturnal cardiorespiratory polygraphy, and OSAS was defined as an apnea/hypopnea index (AHI) ≥5.
Results: The prevalence of high OSAS risk was similar in patients without and with polygraphy (76% vs 68%, p = 0.17). Among these last subjects who underwent polygraphy 50% had OSAS. The prevalence of OSAS was significantly higher in patients with, than in those without, F2–F4 fibrosis (72% vs 44%, respectively; p = 0.04). After correction for confounders, significant fibrosis was associated with mean oxyhemoglobin saturation (SaO2) levels <95% (OR 3.21, 95% CI 1.02–7.34; p = 0.04). Similarly, the prevalence of OSAS was slightly higher in patients with, than in those without, carotid plaques (64% vs 40%; p = 0.08). After correction for confounders, the association between carotid plaques and time spent with SaO2 <90% (T90) >1% was maintained (OR 6.30, 95% CI 1.02–12.3; p = 0.01).
Conclusions: In NAFLD patients without morbid obesity OSAS was highly prevalent and indexes of oxygen saturation were independent indicators of the severity of liver fibrosis and of carotid atherosclerosis risk. These data, if further validated, could suggest to look for OSAS in all NAFLD patients, considering OSAS as a potential additional therapeutic target for NAFLD.

P1014
ASSESSING BIAS IN A MAGNETIC RESONANCE IMAGING METHOD FOR MEASUREMENT OF LIVER FAT FRACTION

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Background and Aims: Most MRI and MRS methods of assessing fatty liver report a ratio of fat protons to water protons. While there can be good correlations between such measures and biopsy observations of liver fat, the two measurements are inherently

measuring different quantities and so bias cannot be assessed directly. The recently cleared (FDA, CE Mark, and TGA) HepaFat-Scan MRI method reports a volumetric fraction of liver tissue that is fat, a measure that can be directly compared with measurements from biopsy histological sections. This study measures the degree of bias of the HepaFat-Scan method against unbiased stereological measurements from biopsies. Stereological analysis (SA) is a method of obtaining unbiased estimates of volume fractions of a phase of material in a 3D matrix from a cross section through the matrix.

Methods: Volumetric fractions of fat in liver tissue of 59 patients (autoimmune hepatitis 3, alcoholic liver disease 2, viral hepatitis 16, NAFLD 10, NASH 17, normal 3, primary sclerosing cholangitis 4, other 4) recruited from the hepatology outpatient clinics at Fremantle and Sir Charles Gairdner Hospitals (Western Australia) were measured by SA of digital images of histological sections of liver biopsies and with non-invasive HepaFat-Scan measurements. Bland–Altman (BA) statistics were used to assess the bias between the two methods of measurement. A steatosis grade for each biopsy was also assessed by an experienced hepatopathologist. ROC curve analysis was used to determine sensitivity and specificity of HepaFat-Scan for predicting steatosis grade.

Results: Figure 1 shows a plot of the volumetric fraction of fat measured by HepaFat-Scan plotted against that measured by SA of biopsy histological sections. The straight line is the line of equivalence (not a fitted regression line). BA analysis indicates a small systematic bias with HepaFat-Scan reporting volumetric liver fat fractions 1.4% higher than SA of biopsy. Sensitivities, specificities and areas under ROC curve for HepaFat-Scan predicting steatosis grade >0 were 97%, 96%, and 0.963; >1 were 100%, 94%, and 0.996; and >2 were 100%, 88%, and 0.971.

Conclusions: The 1.4% bias between HepaFat-Scan and SA of biopsy is not clinically significant since it represents less than 5% of the overall range of fat fractions measured. The observed high sensitivities and specificities indicate that overall accuracy of HepaFat-Scan is sufficient for clinical patient management.

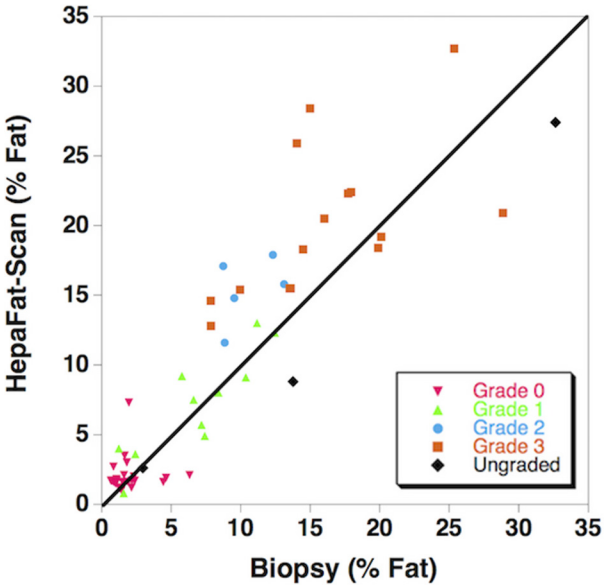

Figure 1.

P1016

FACTORS ASSOCIATED WITH THE DEVELOPMENT OF FATTY LIVER OR GLUCOSE INTOLERANCE

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Background and Aims: Non-alcoholic fatty liver (NAFLD) is a phenotype of the metabolic syndrome in the liver; insulin resistance is the key mechanism of the metabolic syndrome. On the other hand, insulin resistance is also further exacerbated by the development of NAFLD. NAFLD and insulin resistance are supposed to influence each other. However, some patients have only either fatty liver (FL) or impaired glucose intolerance (IGT), although the other one is likely to develop over time. We hypothesized that there are possibly any differences between cases with FL first followed by IGT and those with IGT first followed by FL.

Methods: We obtained clinical and laboratory data from Japanese subjects (3431 men and 1997 women; median age, 47 years old) who serially underwent annual health checkups with ultrasonography in 2009 and 2010 in Junpukai Health Maintenance Center. IGT was defined as fasting plasma glucose level ≥100 mg/dL or use of medications for diabetes mellitus. We analyzed cases with either FL or IGT to determine the factors associated with the development of the other one using logistic regression analysis. We also made a comparison between cases with FL first followed by IGT and those with IGT first followed by FL using Fisher's exact test or Welch's t test.

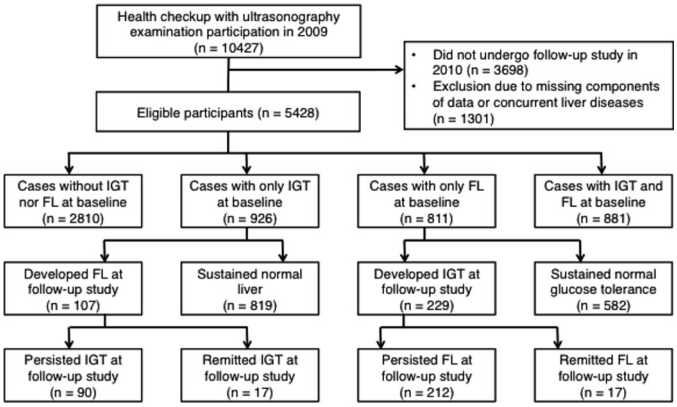

Figure: Inclusion and exclusion flow chart.

Results: In cases with IGT at baseline, 107 out of 926 (12%) newly developed FL; obesity (OR, 2.85; 95% CI, 1.74–4.58) and dyslipidemia (OR, 1.68; 95% CI, 1.07–2.59) were directly, and habitual exercise (OR, 0.61; 95% CI, 0.40–0.91) was inversely associated with the development of FL. Meanwhile, in cases with FL at baseline, 229 out of 811 (28%) newly developed IGT; dyslipidemia (OR, 1.41; 95% CI, 1.03–1.93), male sex (OR, 1.84; 95% CI, 1.22–2.86), and age (1-year increments; OR 1.02; 95% CI, 1.01–1.04) were associated with the development of IGT. In comparison between the cases with IGT first followed by FL and the cases with FL first followed by IGT, the former were significantly elder (51 years old vs. 47 years old, $P = 0.012$) and the latter had significantly higher prevalence of obesity (29% vs. 53%, $P < 0.001$) and dyslipidemia (36% vs. 50%, $P = 0.032$) at baseline.

Conclusions: Obesity and dyslipidemia possibly contribute especially to the development of FL, while aging does to that of IGT.

P1017

CIRCULATING SCLEROSTIN AND DICKKOPF-1 IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: There is evidence for possible bidirectional interplay between hepatic and bone metabolism. The primary aim of this study was to evaluate the serum sclerostin and dickkopf (DKK)-1 levels in patients with nonalcoholic fatty liver disease (NAFLD), untreated for metabolic bone disease, and their association with the disease severity.
Methods: Thirteen and 14 patients with biopsy-proven nonalcoholic simple steatosis (SS) and steatohepatitis (NASH), respectively, and 20 controls of similar gender, age, body mass index (BMI) and waist circumference (WC) were consecutively enrolled. Serum sclerostin, DKK-1, bone-specific alkaline phosphatase (BALP), carboxy-terminal telopeptides of Type I collagen (CTX), 25-hydroxy vitamin D [25(OH)D] and insulin, as well as standard biochemical and hematologic tests were measured. Lumbar spinal (LS) dual energy X-ray absorptiometry (DXA) was also performed.
Results: There was a trend towards decrease in serum sclerostin levels from the controls (76.1±6.8) to SS (53.5±6.4) and NASH (46.0±8.1 pmol/l) patients (p = 0.009); in adjusted pairwise comparisons (Bonferroni correction), sclerostin was significantly higher in the controls than NASH patients (p = 0.012), whereas not statistically different between controls and SS (p = 0.091), or SS and NASH patients (p = 1.0). Serum DKK-1 did not differ between groups (p = 0.135), though it showed a trend for U-shaped distribution (controls 35.8±2.8; SS 27.3±2.9; NASH 36.8±4.4 pmol/l). There was not between group differences in age, BMI, WC, BALP, CTX, 25(OH)D, and LS DXA, whereas liver function tests, insulin and insulin resistance, as expected, were progressively increased from the controls to SS and NASH. After adjustment for gender, BMI, age, log(alanine aminotransferase), CTX, white blood cells and erythrocyte sedimentation rate, between group differences remained significant for sclerostin, and non-significant for DKK-1. Regarding specific histological lesions, DKK-1 levels were marginally lower in NAFLD patients with lower (≤33%) steatosis grade (27.7±3.1) than those with steatosis >33% (38.8±4.7 pmol/l; p = 0.049). No statistically significant differences of sclerostin or DKK-1 were observed in fibrosis grade, portal or lobular inflammation or ballooning.
Conclusions: Serum sclerostin levels were lower in NASH patients compared to the controls, whereas similar DKK-1 levels were observed between groups. These findings warrant further research.

P1018

SIGNIFICANT CORRELATION BETWEEN VISCERAL FAT AREA AND CONTROLLED ATTENUATION PARAMETER

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Background and Aims: This study is designed to investigate the relationship between controlled attenuation parameter (CAP) and visceral fat area (VFA).
Methods: A total of 304 consecutive patients who underwent both of transient elastography and abdominal fat CT during regular health check up at one tertiary center in Korea were enrolled prospectively. To obtain VFA, the visceral and peripheral adipose regions were calculated according to the intervertebral position of L4–5. Clinical, metabolic measurement, and laboratory data were also evaluated. In this study, significant steatosis is defined when CAP value is ≥250 dB/m.
Results: The mean age (165 male, 139 female) was 56.5 years and mean body mass index (BMI) was 24.1 kg/m^2. Twenty-two patients (7.2%) had diabetes mellitus and 44 (14.5%) had hypertension. In univariate analysis, the patients with significant hepatic steatosis had higher BMI, waist/hip ratio, VFA, peripheral fat area, fasting glucose level, triglyceride (TG) level, and alanine aminotransferase (ALT) levels (all $P < 0.05$). In multivariate analysis, VFA (odds ratio [OR] 1.010; 95% confidence interval [CI] 1.001–1.019; $P = 0.028$) and TG (OR 1.006; 95% CI 1.001–1.011; $P = 0.022$) were selected as independent risk factor for significant hepatic steatosis. When the study population was stratified into three groups (VFA ≤100 cm^2, VFA 100.1–200 cm^2, VFA >200 cm^2), patients with a higher VFA were at a greater risk of significant hepatic steatosis with OR 4.838 (P < 0.001; 95% CI 2.912–8.039) for VFA 100.1–200 cm^2; OR 7.474 (P < 0.001; 95% CI 2.462–22.693) for VFA >200 cm^2. In sub analysis of 110 patients with BMI <23 kg/m^2, only VFA was significantly related with hepatic steatosis (OR 1.006; 95% CI 1.001–1.011; P = 0.022).
Conclusions: Our data demonstrated that VFA was significantly related with significant hepatic steatosis assessed by CAP, suggesting that surveillance of hepatic steatosis is needed for patients with central obesity.

P1019

THE IMPACT OF METABOLIC SYNDROME ON ALT LEVELS AMONG THE LARGE MULTIETHNIC COHORT OF NORTH AMERICAN PATIENTS WITH CHRONIC HEPATITIS B INFECTION ENROLLED IN THE HEPATITIS B RESEARCH NETWORK (HBRN)

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Background and Aims: Due to rise in obesity, metabolic syndrome (MS) is prevalent in western countries affecting about 25% of adults in United States. MS is associated with adverse liver disease outcomes including disease progression and hepatocellular carcinoma (HCC). However, the impact of MS on liver disease markers among patients with chronic hepatitis B infection (CHB) living in North America (NA) have not been well studied. We sought to determine the prevalence of MS and its influence on ALT levels at cohort entry and change in ALT over time in a large multi-ethnic NA cohort of patients infected with HBV.
Methods: Adult patients with CHB (HBsAg+ >6 months) who were not pregnant and without liver decompensation, HCC, liver transplant, HIV or current antiviral therapy from 21 US/Canada centers were evaluated. MS was defined as presence of at least 3 of the 5 standard criteria including high population-based waist circumference, blood pressure, glucose and triglyceride levels, and low HDL. Data were assessed at baseline and ALT and HBV DNA were followed over a median of 2.3 years.
Results: 973 patients were included in the analysis: median age 43 yrs, 48% female, 73% Asian (12% black, 11% white), and 82% were born outside US/Canada (28% migrated >20 yrs ago). Participants had a mean of 3 ALT measurements. MS was present in 20% and

those with MS were older (median age 55 vs 40 yrs), more likely to be male (59% vs 51%) black (20% vs 10%), and have HBV DNA <20,000 IU/mL (69% vs 54%), but less likely to be HBeAg-positive (14% vs 29%). In multivariable analysis adjusted for age, gender, race, alcohol use, HBV DNA, and HBeAg, ALT levels at baseline were 1.13 times higher in those with MS than those without MS (log_2 ALT estimate 0.17, 95% CI 0.001–0.35, p = 0.049). However, when controlling for age, gender, race at baseline and HBV DNA levels over time, MS had no significant effect on change in ALT levels over time (log_2 ALT estimate 0.08, p = 0.1); this result was independent of baseline ALT and constant over time.
Conclusions: In this large NA multi-ethnic cohort with chronic HBV, MS was prevalent and was associated with higher baseline ALT levels. Although MS did not influence change in ALT over short term, longer follow-up maybe needed to better characterize the full impact of MS on ALT over time. These results highlight importance of interventions to reduce ALT through weight loss and viral suppression especially among at-risk ethnic minorities and older patients.

P1020
EFFECTS OF URSODEOXYCHOLIC ACID AND PIOGLITAZONE LONG THERAPY ON HEPATOCYTES CHANGES IN NASH PATIENTS
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Background and Aims: The aim of this study was to investigate the effects of ursodeoxycholic acid (UDCA) and pioglitazone long therapy on electronomicroscopic, morphometrical and cytogenetic changes of hepatocytes and insulin resistance (IR) in NASH patients.
Methods: In 27 NASH patients [age 47.3±6.9 yr; body mass index (BMI) 33.9±3.5 kg/m^2] and 12 healthy volunteers [age 44.3±5.5 yr; BMI 24.2±2.8 kg/m^2] obesity and IR were determined by BMI and HOMA-IR. Circulating insulin levels were measured by the immuno-assay method. Electron-microscopic, morphometrical, cytogenetic examinations of hepatocytes and hepatocyte nuclei were investigated. 15 NASH patients were treated with 10–15 mg/kg/day of UDCA (group I), 12 NASH patients were treated with 10–15 mg/kg/day of UDCA + pioglitazone 15–30 mg/day for two years (group II).
Results: After therapy, the condensed chromatin content was decreased in hepatocyte nuclei in group II (p < 0.05). The quantity of the pathologically changed nuclei was decreased in hepatocytes more than 2.6 times and 1.3 times (p < 0.05) in groups I and II vs. patients before treatment. In group II the area of the hepatocytes profile (AHP) was increased – up to 176.40±13.08 μm^2 vs. 128.73±11.74 μm^2 (p = 0.01) before treatment and vs. 182.17±2.54 μm^2 (p > 0.05) in control; in group I – 138.84±11.76 μm^2 vs. 129.25±11.03 μm^2 (p > 0.05) before treatment and vs. 182.17±2.54 μm^2 (p = 0.03) in control. After therapy, one patient (8.33%) in group II and eight (53.33%) in group I had an AHP from 50.0 to 150.0 μm^2 vs. 10 (83.3%) and 12 (80.0%) patients accordingly before treatment; in healthy volunteers AHP ranged from 100.0 to 250.0 μm^2 (85.42%). The area of the nuclear profile of NASH patients in group II was increased – up to 32.45±2.89 μm^2 vs. 21.52±2.17 μm^2 (p = 0.03) and vs. 35.32±0.60 μm^2 in control (p > 0.05), in group I – 25.86±2.45 μm^2 vs. 21.83±2.15 μm^2 (p > 0.05) before treatment and vs. 35.32±0.60 μm^2 in control (p = 0.04). Compared with group I, HOMA-IR increased in group II after UDCA + pioglitazone therapy (p < 0.02).
Conclusions: The UDCA + pioglitazone combination in long-term use for two years has metabolic effects with beneficial cytoprotective properties and improves electronomicroscopic, morphometrical and cytogenetic markers of the hepatocytes and hepatocyte nuclei in NASH patients.

P1021
EFFECTS OF URSODEOXYCHOLIC ACID AND PIOGLITAZONE LONG THERAPY ON NASH COURSE
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Background and Aims: Non-alcoholic steatohepatitis (NASH) occurs during the progression of non-alcoholic fatty liver disease. The aim of this study was to investigate the effects of ursodeoxycholic acid (UDCA) and pioglitazone on lipid metabolism, markers of insulin resistance (IR), angiogenesis and fibrosis and histological liver changes in NASH patients.
Methods: In 43 NASH patients [age, 47.2±6.9 yr; body mass index (BMI), 34.4±3.6 kg/m^2] and 12 healthy volunteers [age, 44.3±5.5 yr; BMI, 24.2±2.8 kg/m^2] obesity and IR were determined by BMI, *dyslipidemia* and HOMA-IR. Circulating insulin, leptin, *fibroblast growth factor basic* (FGF-b), vascular endothelial growth factor (VEGF), and type IV collagen levels were measured by the immuno-assay method. Histologic liver changes and morphometrical parameters of the hepatocytes were determined. 16 NASH patients had been without medical treatment (group I), 15 NASH patients were treated with 10–15 mg/kg/day of UDCA for two years (group II), 12 NASH patients were treated with 10–15 mg/kg/day of UDCA+pioglitazone 15–30 mg/day for two years (group III).
Results: In patients of group I a statistically significant increase of HOMA-IR (p = 0.002) and circulating triglycerides (p = 0.009), low density lipoprotein cholesterol (LDLC) (p = 0.007), leptin (p = 0.003), FGF-b (p = 0.007), VEGF (p = 0.005), and type IV collagen (p = 0.01) were noted; this associated with an increase of the histological parameters: steatosis (p = 0.01), inflammation (p = 0.04), ballooning (p = 0.03), and fibrosis (p = 0.01). After treatment, in patients of groups II and III we observed reductions in circulating triglycerides (p = 0.02 vs. p = 0.007), LDLC (p = 0.04 vs. p = 0.009), type IV collagen (p = 0.04 vs. p = 0.01) and histological parameters: steatosis (p = 0.04 vs. p = 0.01), inflammation (p = 0.03 vs. p = 0.009), ballooning (p = 0.03 vs. p = 0.01). Compared with groups I and II, in the UDCA+pioglitazone group HOMA-IR (p = 0.01), circulating leptin (p = 0.02), FGF-b (p = 0.03), and VEGF level (p = 0.01) decreased, which were associated with a reduction in fibrosis (p = 0.02).
Conclusions: The UDCA+pioglitazone combination for two-year use has metabolic, angiogenic, anti-inflammatory and antifibrotic effects in NASH patients and reduces progression of NASH course.

P1022
FIBRO SCAN AND COGNITIVE DYSFUNCTION IN OBESE INDIVIDUALS WITH METABOLIC RISK FACTORS
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Background and Aims: *Background:* The metabolic syndrome (MS) may be associated with cognitive impairment and increase in oxidative stress. *Aim:* To document the association between MS and cognitive impairment in metabolically healthy obese (MHO), and in metabolically unhealthy obese (MAO) individuals.
Methods: 60 obese individuals aged 49±10 years (52% male) were enrolled. Obesity was defined as BMI >30 kg/m^2. MS was defined according to ATP III guidelines. Obese individuals were divided into two groups: group 1, MHO (≤2 components of MS), and group 2, MAO (>2 components of MS). Cognitive dysfunction was determined by MOCA – Montreal cognitive assessment score (MOCA 11–21, dementia; MOCA 19–25, mild cognitive impairment;

or MOCA 25–30, normal test score). Fibro scan (XL probe), Inflammation (CRP), pro oxidants (MDA), antioxidants activity (PON, GSH) and insulin resistance (HOMA) were measured.
Results: Of the 30 MAO individuals, 13% developed dementia, 51% had mild cognitive impairment, and 36% had normal cognitive score as compared to 3%, 7%, and 90% in the MHO group respectively. There was a significant difference in liver stiffness (Normal <6.0 kPa, 7±3 vs. 5.2±2.7 kPa, $p < 0.001$), liver fat measurement (CAP normal <270 dB/m, 337±51 vs. 280±20, $p < 0.001$), CRP (9±6 vs. 7±6, $P < 0.001$), MDA (5.9±0.8 mM vs 4.3±0.7 mM, $P < 0.01$), Paroxonase (0.06±0.01 mM vs. 0.12±0.08 mM, $P < 0.001$), GSH (27.2±2.4 vs. 28.4±2.3, $P < 0.03$), and HOMA (6±5.5 vs. 2.5±1, $P < 0.02$) levels between the two groups respectively. Correlations between cognitive score and components of MS was strong with blood pressure ($r = -0.252$), abdominal girth ($r = -0.26$), and liver stiffness measurements ($r = -0.256$). Multivariate analysis accounting for confounders showed that Fibro scan ($T = 2.5$), abdominal girth ($T = -2.1$, $P < 0.04$) and age ($T = -3.0$, $P < 0.009$) were the most powerful predictors of cognitive dysfunction.
Conclusions: There is a significant cognitive impairment and increase in oxidative stress in MAO (50%). Whether treatment of MAO would improve cognitive impairment remains to be determined.

P1023
QUANTIFICATION OF LIVER, PANCREAS, KIDNEY AND VERTEBRAL BODY MRI-PDFF IN NONALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: A recent magnetic resonance technique – magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF) –gives quantitative information of fat deposition in liver and has been shown to have a good correlation with both magnetic resonance spectroscopy and liver biopsy. Apart from this, it has a potential for quantifying the fat fraction in other tissues. The aim of the present study was to determine liver, pancreas, kidney and vertebral fat deposition in nonalcoholic fatty liver disease (NAFLD) patients by MRI-PDFF and to evaluate the relationships among them.
Methods: A total of 41 biopsy proven NAFLD patients underwent MRI-PDFF with IDEAL-IQ. MRI protocol included T1-independent volumetric multi-echo gradient-echo imaging with T2* correction and spectral fat modeling. The MR examinations were performed on a 1.5 HDx MRI system. MRI-PDFF measurements were obtained from liver, pancreas, renal cortex and sinus and vertebral body. Liver biopsy specimens were retrieved from the archives and evaluated by one pathologist according to NASH-CRN.
Results: The median age of the patients was 47 years. The median interval between liver biopsy and MRI examination was 16 days. Mean liver, pancreas, renal cortex, renal sinus, T12 and L1 vertebral body MRI-PDFF was 18.7%, 5.7%, 1.7%, 51%, 43.2% and 43.5%, respectively. A close correlation was observed between liver MRI-PDFF and histologic steatosis in terms of the quantification of both percentage of steatotic hepatocytes ($r_s = 0.809$, $p < 0.001$) and steatosis grade ($r_s = 0.848$, $p < 0.001$). However, no correlation between either liver MRI-PDFF or histological steatosis, and other organ MRI-PDFFs was observed. A good correlation between pancreas and vertebral body MRI-PDFFs, and pancreas and renal sinus MRI-PDFFs was observed. Diabetic patients had higher average pancreas MRI-PDFF compared to non-diabetics (12.2% vs. 4.8%; $p = 0.028$).
Conclusions: Based on the present study, pancreas PDFF is well correlated with vertebral body and renal sinus fat PDFF independent of liver PDFF. Diabetes increases pancreas PDFF in NAFLD patients.

P1024
POPULATION, PRESCRIPTION AND EFFECTIVENESS OF ADOMET TREATMENT FOR INTRAHEPATIC CHOLESTASIS IN CHRONIC LIVER DISEASE DUE TO NON ALCOHOLIC LIVER DISEASE (IHC IN NALD) IN THREE POST MARKETING SURVEILLANCE STUDIES IN RUSSIA, UKRAINE AND INDIA

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Background and Aims: Three non-interventional, prospective, multi-centre post-marketing observational surveillance studies (PMOS) in Russia, Ukraine and India including 525 subjects with intrahepatic cholestasis in chronic liver disease due to *non-alcoholic liver disease* (IHC in NALD) were performed. In these studies, AdoMet (S-Adenosyl-L-methionine) was prescribed according to current medical practice. This research provides insight into prescription mode, patient population and effectiveness.
Methods: In Russia, subjects received AdoMet start-up therapy (iv or im 400 or 800 mg) for 2 weeks and then oral maintenance therapy for 4 weeks (400 to 1600 mg tablets). In India, all subjects started on tablets, with effectiveness evaluation at baseline and around 42 days. In Ukraine, subjects started on either injections or tablets. Measurements were taken at two weeks and two months.
Results: In total, 98 subjects were included in Russia, 185 in Ukraine and 242 subjects in India. In India, 44% of subjects were on 1200 mg tablets and 55% on 800 mg tablets. In Russia, 47% of subjects were on 800 mg IV, 53% on 400 mg IV. In Ukraine, 46% of subjects were on 400 mg IV, 20% on 800 mg IV, 23% on 800 mg tablets and 11% on other.
63% was male, with mean age 45 years. Steatosis was the most often indicated medical history (90% in Russia, 57% in India and 25% in Ukraine), followed by chronic hepatitis (70% in Ukraine, 26% in India and 17% in Russia).
Table 1 provides the reductions in laboratory values and symptoms.
In Russia and Ukraine, the majority of laboratory values and symptoms were significantly better at end of treatment than at switch from start-up injection therapy to oral maintenance therapy.
Conclusions: Large reductions in GGT, AST, ALT, total bilirubin, conjugated bilirubin and ALP and symptoms were found with Adomet treatment in patients with IHC due to NALD for each country.

Table 1.

	Ukraine (N = 185)			Russia (N = 98)			India (N = 242)		
	BL	End	% Reduction	BL	End	% Reduction	BL	End	% Reduction
ALP (IU/L)	242	127	47%	174	110	37%	187	143	23%
ALT (IU/L)	130	45	65%	65	36	45%	90	54	40%
AST (IU/L)	87	35	60%	55	30	46%	90	53	41%
Conjugated bilirubin (μmol/L)	23	9	59%	7	5	28%	14	10	25%
GGT (IU/L)	113	61	46%	103	52	50%	96	64	33%
Total bilirubin (μmol/L)	46	20	57%	21	16	23%	31	22	30%
Fatigue (n)	167	87	48%	73	12	84%	179	76	58%
Jaundice (n)	129	27	79%	9	1	89%	114	49	57%
Pruritus (n)	204	28	73%	33	4	88%	92	40	57%

BL, baseline.

P1025

INCREASING BURDEN OF NASH/CRYPTOGENIC CIRRHOSIS IN CONTRIBUTION TO END-STAGE LIVER COMPLICATIONS IN ASIA-PACIFIC

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is increasingly recognised as a leading cause of liver morbidity and mortality globally. The prevalence of NAFLD is rapidly rising due to the epidemic surge in obesity and Diabetes Mellitus. NAFLD consists of a spectrum including non-alcoholic steatohepatitis (NASH), and 20% of patients with NASH progress to cirrhosis. Studies indicate that the majority of hepatocellular carcinoma (HCC) in cryptogenic cirrhotic patients is associated with prior NAFLD or other features of the metabolic syndrome. However, its disease burden in the Asia-Pacific region remains unknown. We aimed to conduct a retrospective analysis of the contribution of NASH/cryptogenic cirrhosis to liver disease burden in a major tertiary care hospital in Singapore.
Methods: A retrospective analysis of data from the National University Hospital Singapore from 1997 to October 2013 was carried out. The objective was to determine the contribution of NASH/cryptogenic cirrhosis over 2 different time periods from 1997 to 2004 and 2005 to 2013. We looked at 2 separate cohorts of patients: those who presented with HCC and those who had undergone liver transplantation surgery for end-stage liver disease.
Results: 806 patients were diagnosed with primary HCC from 1997 to October 2013. Of these patients, 72 (9%) had been established with NASH/cryptogenic cirrhosis. For the HCC cohort from the period of 1997 to 2004, 2 out of 42 patients (5%) were diagnosed with NASH/cryptogenic cirrhosis and subsequently 70 out of 764 patients (10%) from 2005 to 2013. For the transplant group, there were 5 patients with NASH/cryptogenic cirrhosis out of 53 patients (9%) who underwent liver transplantation from 1997 to 2004 and 9 NASH/cryptogenic cirrhotic patients out of 76 (12%) who did so from 2005 to 2013. Majority of the patients had underlying Hepatitis B cirrhosis.
Conclusions: These figures demonstrate a definite increasing burden of NASH/cryptogenic cirrhosis over the years in end-stage liver complications. NASH will likely transcend viral hepatitis as the main aetiology of liver disease burden in the Asia-Pacific region in the near future and this underlies the imperative need to address this ongoing epidemic.

P1026

THE EVALUATION OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND ITS ASSOCIATE FACTORS IN PSORIASIS PATIENTS USING ULTRASONOGRAPHY AND THE CONTROLLED ATTENUATION PARAMETER (CAP) MEASURED WITH TRANSIENT ELASTOGRAPHY

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Background and Aims: Psoriasis is linked to metabolic syndrome and nonalcoholic fatty liver disease (NAFLD). Fatty liver can be diagnosed by ultrasonography (US) but sensitivity is reduced when steatosis <30%. Controlled attenuation parameter (CAP) measures liver fat base on ultrasound signal acquired by transient elastography (TE, Fibroscan®). We aimed to estimate the prevalence of fatty liver, to identify factors associated with fatty liver and to assess the performance of CAP for assessing fatty liver in psoriasis patients.
Methods: A cross-sectional study was performed in psoriasis patients without liver diseases. Subjects underwent US and CAP measured by TE. Fatty liver appeared as an increased echogenicity on US. Grading of fatty liver was classified as mild, moderate or severe degree. Performance of TE required 10 successful shots, IQR <30% and success rate >60%. Factors with p-value of univariate analysis <0.20 were simultaneously entered logistic regression model. An optimal cut-off of CAP was determined using Youden index. Diagnostic parameters were estimated. P-value <0.05 was taken as statistical significance.
Results: 168 psoriasis patients were enrolled. TE failure occurred in 3 patients. Seventy-five patients (45.5%) were males with age of 49.22 (14.0) years. Diabetes, hypertension, dyslipidemia and metabolic syndrome were found in 31 (18.8%), 55 (33.3%), 88 (53.3%) and 83 (50.7%) patients. Fatty liver was detected in 105 (63.6%)

Table (abstract P1026): Univariate and multivariate analysis of factors relating to the presence of fatty liver

	Normal liver	Fatty liver by US	p value	Univariate analysis			Multivariate analysis		
				OR	95% CI	p-value	OR	95% CI	p-value
Number (%)	60 (36.4%)	105 (63.6%)							
Age (years) [a]	45.67 (16.6)	51.25 (11.9)	0.013	1.03	1.01–1.05	0.015			
Male [b]	20 (26.7%)	55 (73.3%)	0.013	2.20	1.14–4.25	0.019			
Diabetes [b]	4 (12.9%)	27 (87.1%)	0.002	4.85	1.61–14.63	0.005			
Hypertension [b]	12 (21.8%)	43 (78.2%)	0.004	2.77	1.32–5.83	0.007	7.94	1.05–60.00	0.045
Dyslipidemia [b]	23 (26.1%)	65 (73.9%)	0.003	2.61	1.36–5.02	0.004			
Metabolic syndrome [b]	11 (13.3%)	72 (86.7%)	0.000	9.72	4.49–21.05	0.000			
PASI score [a]	2.53 (2.36)	3.25 (2.9)	0.08	1.12	0.98–1.28	0.104			
BMI (kg/m^2) [a]	21.23 (2.6)	26.78 (4.4)	0.000	1.70	1.44–2.00	0.000	1.63	1.13–2.37	0.009
<22.9 kg/m^2 [b]	49 (76.6%)	15 (23.4%)	0.000						
23–25 kg/m^2 [b]	4 (14.3%)	24 (85.7%)		19.6	5.87–65.48	0.000	71.02	6.51–774.43	0.000
>25 kg/m^2 [b]	7 (9.6%)	66 (90.4%)		30.8	11.67–81.27	0.000	35.32	3.64–343.14	0.002
Waist circumference (cm) [a]	77.66 (7.3)	92.40 (11.4)	0.000	1.1 9	1.125–1.256	0.000			
ALT (u/l) [a]	33.67 (16.6)	45.51 (25.0)	0.000	1.04	1.012–1.060	0.003			
GGT (u/l) [a]	28.42 (24.4)	49.10 (47.2)	0.000	1.02	1.007–1.042	0.005			
HOMA [a]	1.60 (1.4)	5.08 (7.3)	0.000	1.95	1.494–2.552	0.000			
LSM (kPa) [a]	4.28 (1.65)	5.83 (3.3)	0.000	1.46	1.165–1.829	0.001			
CAP (dB/m) [a]	182.93 (41.8)	278.10 (52.1)	0.000	1.04	1.03–1.06	0.000	1.04	1.02–1.05	0.000

[a] Mean (SD), [b] n (%).

patients. Fifty-six (33.9%), 38 (23.0%) and 11 (6.7%) patients had mild, moderate and severe fatty liver. Mean CAP and liver stiffness measurement in normal, mild, moderate and severe fatty liver were 182.93 (41.8), 247.96 (43.0), 307.86 (39.1), 329.27 (32.5) dB/m, and 4.28 (1.7), 5.02 (3.4), 6.05 (2.1), 9.16 (4.3) kPa. After adjusting for hypertension and BMI, CAP was associated with fatty liver with OR 1.05 (95% CI 1.03–1.07, $p < 0.001$). The optimal cutoffs of CAP for mild and severe fatty liver were 238 and 315 dB/m yielding the Areas Under ROC curves (AUROC), sensitivity and specificity of 0.92, 79.05%, 95.00% and 0.90, 81.82%, 88.31%. Hypertension (OR 7.94, 95% CI: 1.05–60.00; $p = 0.045$), BMI 23–25 kg/m^2 (OR 71.02, 95% CI: 6.51–774.43; $p = 0.000$) and BMI >25 kg/m^2 (OR 35.32, 95% CI: 3.64–343.14; $p = 0.002$) were associated with fatty liver.

Conclusions: Sixty-four percents of psoriasis patients have fatty liver by US. Hypertension, overweight, obesity and CAP are related to fatty liver. CAP using TE can be used to evaluate fatty liver in psoriasis patients.

P1027

DO LEPTIN PROFILE AND INSULIN RESISTANCE FAVOR OXIDATIVE STRESS AND DISEASE SEVERITY IN NON-ALCOHOLIC FATTY LIVER DISEASE?

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Background and Aims: Leptin (L) promotes oxidative stress (OS) and the fibrogenesis. We aimed to evaluate the association between L levels, OS parameters, apoptosis and histopathological findings in Non alcoholic fatty liver disease (NAFLD) with and without IR.

Methods: Fifty-eight NAFLD patients were studied (M/F:31/27 mean age 47.4±6.3). Thirty-six NAFLD patients with IR were compared with 22 subjects without IR. For the determination of oxidative stress, malondialdehyde (MDA), and superoxide dismutase (SOD) activities were measured in serum and in tissue specimens. Glutathione (GH) was measured in tissue homogenates. Nitric oxide (NO) and TNF-alpha receptor (TNF-sRp55) levels were measured in serum. For apoptotic activity immunohistochemistry was performed for caspase-3 and 8, transcription nuclear factor kB (NF-kB), and antiapoptotic Bcl-2 protein. Multivariate regression analysis and ROC curve were used to identify the independent predictors for NASH.

Results: In bivariate analysis serum leptin levels didn't show any significant correlation with steatosis grade, necroinflammatory grade and stage. In linear regression analysis serum NO, tissue MDA, caspase-3, caspase-8, and stage were independently associated with increased leptin levels. HOMA-IR index correlated positively with the necroinflammatory grade, the stage, caspase-3 and 8 levels. Patients with IR had significantly higher steatosis grade, necroinflammatory grade, stage, caspase-3, caspase-8, TNF-sRp55, serum NO, tissue MDA, GH and serum leptin levels than those without IR. Using serum leptin levels the ROC curve for distinguishing between non-alcoholic steatohepatitis (NASH) and simple steatosis didn't show any respective sensitivity and specificity (AUROC=0.46). In multivariate regression analysis increase of tissue MDA, serum NO levels, caspase-3 and caspase-8 levels were risk factors for NASH and increase of leptin activity had preventive effect against NASH (OR:0.048; %95 CI:0.006–0.68, $p = 0.04$).

Conclusions: IR in NAFLD is associated with increased oxidative stress, hepatocyte apoptosis and histopathological disease severity. In patients with IR oxidative stress parameters, apoptotic caspase levels and stage were independently associated with increased L levels and increased L activity showed preventive effect against NASH. These data indicate that NAFLD patients with IR may have increased risk for disease progression and leptin may have a preventive effect against oxidative stress, apoptosis and liver fibrosis.

P1028

IMPAIRED AEROBIC CAPACITY AND CARDIAC AUTONOMIC CONTROL IN SEDENTARY POSTMENOPAUSAL WOMEN WITH NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Background and Aims: Aerobic capacity indexes and cardiac autonomic control have been associated with cardiovascular risk in general populations. It is important to investigate whether these parameters are also impaired in Nonalcoholic fatty liver disease (NAFLD) patients as compared with healthy controls. The aim of this study was to compare the aerobic capacity and cardiac autonomic control between postmenopausal women with or without NAFLD (CTRL) and investigate this relation according to hepatic severity.

Methods: Thirty-seven physically inactive postmenopausal women (age: 55.8±1.4 years; BMI: 33.0±0.7 kg/m^2) biopsy-proven NAFLD and twenty-four CTRL (age 55.8±0.7 years; BMI: 29.0±1.0 kg/m^2) performed a cardiopulmonary exercise test to assess the aerobic capacity indexes [i.e., time at ventilatory anaerobic threshold (VAT), time at respiratory compensation point (RCP), time to exhaustion and maximal oxygen uptake (VO_2max)] and heart rate recovery of the first and second minutes after the end of the test (i.e., HRR1min and HRR2min).

Results: NAFLD patients showed lower time to exhaustion (11.2±0.3 *vs.* 12.5±0.4 min, $p = 0.026$) and time at RCP (8.9±0.4 *vs.* 10.1±0.4 min, $p = 0.047$) when compared to CTRL. Additionally, time at VAT presented a tendency to be lower in NAFLD patients when compared with CTRL (4.6±0.2 *vs.* 5.2±0.3 min, $p = 0.067$). The HRR1min and HRR2min showed also a tendency to be lower in NAFLD patients compared with CTRL ($p = 0.05$ and $p = 0.059$, respectively). No significant changes were noted in the time at RCP between fibrosis degree 0/1/2 in comparison to degree 3/4 (8.5±2.5 *vs.* 10.2±1.8 min, $p = 0.078$). HRR2min showed a tendency to be higher in fibrosis degree 0/1/2 when compared to fibrosis degree 3/4 ($p = 0.093$).

Conclusions: NAFLD patients showed lower aerobic capacity and an impaired cardiac autonomic control, which are indexes independently associated with risk of cardiovascular events. However, these findings require future investigations in large population-based studies.

P1029

RACIAL-ETHNIC DISPARITIES IN LIVER DISEASE MORTALITY IN THE UNITED STATES

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Background and Aims: Chronic liver disease and cirrhosis was the 12th leading cause of death in the U.S. in 2011 and the mortality rate varies among racial-ethnic groups. Age-adjusted liver disease mortality per 100,000 was 32.3 for Hispanics compared with 21.6 for both non-Hispanic whites and non-Hispanic blacks (http://wonder.cdc.gov/). We examined factors explaining racial-ethnic differences in liver disease mortality in a U.S. population-based prospective study.

Methods: Data were analyzed from 17,681 adult non-Hispanic white, non-Hispanic black, and Mexican American participants in

the third U.S. National Health and Nutrition Examination Survey, 1988–1994. Participants were followed through 2011 for mortality as identified by death certificate diagnoses. Hazard rate ratios (HR) for mortality were calculated using Cox proportional hazards regression to adjust for factors associated with both race-ethnicity and mortality.

Results: Mexican Americans had adverse measures of socio-economic status and were more likely to have obesity and diabetes compared with non-Hispanic whites at baseline. During up to 23 years of follow-up, there were 201 deaths with liver disease, including viral hepatitis and primary liver cancer, as the underlying or a contributing diagnosis. In age- and sex-adjusted analysis, liver disease mortality was twice as high among Mexican Americans compared with non-Hispanic whites, while there was no statistically significant difference for non-Hispanic blacks (Table). Adjustment for hepatitis B or C infection or for non-alcoholic fatty liver disease (NAFLD) risk factors (BMI, waist-to-hip ratio, diabetes) attenuated the association of Mexican American ethnicity with higher liver disease mortality. With adjustment for socioeconomic status (SES; education, income) and health insurance, Mexican American ethnicity was not statistically significantly associated with higher liver disease mortality. Results were similar with simultaneous adjustment for all of the above factors. Limiting analyses to deaths with liver disease as underlying cause had little effect on relationships.

Table: Liver disease mortality HR (95% CI) compared with non-Hispanic whites

Adjusted for	Non-Hispanic blacks	Mexican Americans
Age, sex	1.6 (1.0–2.5)	2.2 (1.3–3.5)
Age, sex, viral hepatitis	1.3 (0.7–2.2)	2.0 (1.2–3.5)
Age, sex, NAFLD risk factors	1.4 (0.8–2.5)	1.9 (1.1–3.4)
Age, sex, SES, insurance	1.2 (0.6–2.1)	1.3 (0.6–2.7)
All factors listed above	0.9 (0.5–1.8)	1.3 (0.6–2.5)

Conclusions: In the U.S. population, lower socio-economic status and lack of health insurance made a greater contribution to higher liver disease mortality among Mexican Americans than did viral hepatitis or NAFLD risk factors.

P1030
THE PRESENCE OF WHITE MATTER LESIONS IS NOT ASSOCIATED WITH NON-ALCOHOLIC FATTY LIVER DISEASE BUT WITH ITS HISTOLOGICAL SEVERITY

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) has been associated with increased cardiovascular risk, including coronary artery disease and cerebrovascular events. No studies however assessed the potential relationship between NAFLD and subclinical cerebrovascular alterations. We tested the correlation between NAFLD and its histological severity with vascular white matter lesions (WML) in patients with biopsy-proven NAFLD and in non steatosic controls.

Methods: The anthropometric, biochemical and metabolic features were recorded in 77 consecutive biopsy-proven NAFLD (Kleiner score), and in 35 controls with normal ALT, without chronic liver diseases, and without ultrasonographic evidence of steatosis. All patients underwent minimental test (MMT) and magnetic resonance assessment of WML. MMT was considered pathologic if <23. WML were classified according to the Fazekas score in absent (0/III), or present (mild I/III; moderate II/III, and severe I/III). For purpose of analyses all controls, as plausible, were considered without NASH and without F2–F4 liver fibrosis.

Results: WML were found in 26% of the entire cohort (29/112), even if of a moderate-severe grade in 5 patients only. The prevalence of WML was similar in NAFLD compared to no NAFLD (27% vs 23%; $p = 0.62$). Age ≥50 yrs, female gender, type 2 diabetes, arterial hypertension, presence of NASH (35% vs 18%, $p = 0.05$) and presence of F2–F4 fibrosis (43% vs 17%, $p = 0.003$) were associated with WML presence ($p \leq 0.01$). At multivariate analysis age >50 yrs (OR 3.44, 95% CI 1.01–11.6, p 0.04), female gender (OR 3.71, 95% CI 1.28–10.7, p 0.01), and F2–F4 fibrosis (OR 3.39, 95% CI 1.17–9.84, p 0.02) were maintained as factors independently associated with WML. When considering NAFLD patients only, we confirmed F2–F4 fibrosis as the only independent predictor of WML (OR 4.24, 95% CI 1.14–15.7, p 0.03). A pathological MMT was found in 10/112 patients (9%) – all of them with NAFLD. Specifically the prevalence of an alterated MMT was 17% in patients with WML and 8% in those without.

Conclusions: The presence of WML is not asociated with NAFLD but with its histological severity. Clinical implications of this issue need to be assessed by longitudinal studies. The ability of MMT to detect subclinical WML was poor.

P1031
COMPARISON OF CLINICAL, BIOCHEMICAL, AND HISTOPATHOLOGICAL PROFILES BETWEEN NAFLD IN ASIAN-INDIANS AND UNITED STATES ADULTS

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver disorders in Asian and Western countries. The epidemiological and demographic character of NAFLD patients differs with geographic variation. Studies across ethnicities in the United States (U.S.) reveal a higher prevalence in Hispanics and African-Americans with limited studies involving Asians. Data suggest that Asian-Indian patients tend to have different characteristics than their counterparts in the West. This study is the first attempt at comparing the characteristics of Asian-Indian and U.S. NAFLD patients.

Methods: A retrospective analysis of clinical, biochemical and histological parameters was performed for 633 Asian-Indian NAFLD patients with 451 U.S. NAFLD patients. Comparisons among the study cohort included clinical (i.e.: age, gender, BMI, diabetes, hypertension, etc.), biochemical tests (i.e.: liver function tests, lipid profile, fasting blood sugar), hepatic ultrasound and hepatic histology.

Results: The majority of U.S. NAFLD patients (82.3%) were over 40 years of age compared to 51.3% of Asian-Indian patients ($\chi^2 = 109.55$; $p < 0.001$). There was significant difference in gender prevalence between U.S. (male 56.3%) and Asian-Indians (male 81.7%) ($\chi^2 = 82.442$; $p < 0.001$). U.S. patients had higher rates of obesity (BMI 32.6 ± 5.3 kg/m^2 versus 26.2 ± 3.4 kg/m^2; $p < 0.001$). With respect to co-morbid conditions, U.S. patients had a higher prevalence of both diabetes and hypertension (diabetes 42.1% vs. 33%, and hypertension 56.8% vs 29.7%, respectively; $p < 0.001$). U.S. NAFLD patients have significantly

higher ALT levels compared to Asian-Indians (ALT 82.78±71.30 versus 53.66±37, p < 0.001). Histological comparison showed that a greater proportion of U.S. patients had more advanced liver disease at time of diagnosis compared to Asian-Indians (Stage 3 fibrosis 10.42% vs. 0%, respectively, and Stage 4 fibrosis 2.66% vs. 0%, respectively, p < 0.001).
Conclusions: Significant differences between Asian-Indian and U.S. NAFLD patients exist. Further study is needed to better understand mechanistically why these differences exist.

P1032
PATIENTS WITH METABOLIC SYNDROME AND NAFLD: ASSESSMENT OF OBESITY AND HEART FIBROSIS DEGREE
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Background and Aims: to investigate level of obesity and heart fibrosis in patients with metabolic syndrome (MetS) and nonalcoholic fatty liver disease (NAFLD).
Methods: The research has included 76 patients, from them 43 patients were with MetS (72.1%, 31 out of 43, with NAFLD) (basic group), 33 patients without MetS (control group). Epicardial fat thickness was evaluated by transthoracic echocardiogram. Besides the above we conducted noninvasive evaluation of the fraction of fibrosis of the myocardium using a new original method – we used echocardiography, then we obtained the resulting images in "jpeg" format and analyzed them using software "Image J v.1.4 (NIH, 2009)". The level of leptin was investigated.
Results: Patients in the basic group had significantly higher epicardial fat thickness than control (4.67±1.7 and 2.66±1.15 mm, p < 0.001). The average volume fraction of fibrosis in the interventricular septum had significant difference in the groups studied (22.6±4.45% in the MetS group and 16.5±3.95% in the controls, p < 0.001). The average level of leptin in the group with MetS and NAFLD was significantly higher in comparison with control (41.89±33.28 vs 17.64±16.87 ng/ml, p < 0.001). Positive correlation was revealed between the level of leptin and weight, the body mass index, the degree of obesity, abdominal obesity, waist circumference, epicardial fat thickness, degree left ventricular hypertrophy (LVH), hepatic and pancreatic steatosis (p < 0.05).
Conclusions: (1) Patients with MetS and NAFLD have epicardial fat thickness significantly higher than control, leptin level is correlated with obesity of the whole body and hearts, which can be a predictor of cardiovascular disease. (2) Relationship has been found between the level of leptin and diseases related to MetS: NAFLD, pancreatic steatosis, LVH. (3) We propose the new method of noninvasive assessment of the volume fibrosis fraction. More strongly pronounced septal fibrosis was found in patients with MetS and NAFLD.

P1033
VITAMIN D DEFICIENCY, NOT VITAMIN D INTAKE WAS INDEPENDENT RISK FACTOR OF ABNORMAL LIVER ENZYME
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Background and Aims: Several reports showed that serum vitamin D deficiency associated with prevalence and progression of liver disease. But it is not uncertain whether vitamin D intake also has pivotal role in serum vitamin D deficiency and abnormal liver enzyme.
Methods: We used the data from Korean National Health and Nutrition Examinations (2008–2011). A total of 27,463 people were included. Vitamin D deficiency was defined as below 20 ng/mL of 25-OH-vitamin D.
Results: Serum vitamin D was positive correlation with body mass index, fasting glucose, and aminotransferase activity. Both vitamin D intake and inhabitable area (urban or rural area) were independent risk factor of serum vitamin D. Although the total amount of vitamin D uptake was lower in rural area and Blue-collar workers compare to urban and White-collar subjects, serum vitamin D was high in rural area. Amount of vitamin D intake did not show any correlation with aminotransferase activity as well as metabolic parameters. Vitamin D deficiency, not vitamin D intake was independent risk factor of abnormal liver enzyme only in obese and blue collar subjects.
Conclusions: Vitamin D deficiency was a risk factor of elevated aminotransferase level and prevalence of metabolic syndrome in obesity. But amount of vitamin D intake did not show any association with metabolic parameters as well as liver chemisty.

P1034
CHARACTERISATION OF THE PREVALENCE AND RISK FACTORS FOR BIOPSY-PROVEN NON-ALCOHOLIC STEATOHEPATITIS AMONG PATIENTS WITH GALLSTONES: A PROSPECTIVE STUDY
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Background and Aims: Whether a link exists between gallstones and non-alcoholic steatohepatits (NASH) remains unclear. Only three studies have reported the prevalence of NASH among gallstones patients showing rates varying widely from 18% to 77%. There are, however, major limitations on these reports due to selection biases as well as the lack of well-defined criteria of definite NASH on liver histology. Therefore, we aimed to prospectively determine the prevalence and risk factors for biopsy-proven definite NASH among unselected patients with gallstones.
Methods: Patients referred for laparoscopic cholecystectomy due to gallstones were offered to participate in the study. Anthropometric and laboratory evaluation, abdominal ultrasound and a liver biopsy during the surgical procedure were performed to each participant. Definite NASH was diagnosed using the Kleiner's histological scoring system.
Results: Two hundred and fifteen consecutive patients with gallstones were included. Prevalence of NASH was 10.2% whereas that of simple steatosis (SS) was 41.4%. Gallstones patients with NASH had a higher mean homeostatic model assessment (HOMA) score than those with SS ($P = 0.015$). Noteworthy, NASH was 2.5-fold more frequent in patients with gallstones who had metabolic syndrome than in those who did not ($P < 0.001$). Fatty liver on ultrasound was observed in 90.9% of gallstones patients with NASH compared with 61.8% of those with SS ($P = 0.044$). Using multivariate logistic regression analysis, increased HOMA score (OR, 3.47; 95% CI, 1.41–8.52; $P = 0.007$) and fatty liver on ultrasound (OR, 23.27; 95% CI, 4.15–130.55; $P < 0.001$) were the only factors independently associated with NASH. The sensitivity and specificity of the combination of the 2 NASH predictors were 83% and 77%, respectively. The area under the ROC curve was 0.897; 95% CI: 0.836–0.958, $P < 0.001$.
Conclusions: The prevalence of NASH among patients with gallstones is lower than estimated previously, but NASH is frequent particularly in those gallstones patients with concurrent metabolic syndrome. The combination of an increased HOMA score with fatty

liver on ultrasound has a good accuracy for predicting NASH in patients with gallstones. Based on these results, we propose that gallstones patients who have preoperative evidence of both insulin resistance and fatty liver on ultrasound are considered to undergo a needle liver biopsy during cholecystectomy for early diagnosis of NASH at high-risk patients.

P1035

ELF TEST IS A RELIABLE NON INVASIVE TEST FOR FIBROSIS IN NAFLD SUBJECTS

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Background and Aims: The identification of fibrosis in patients with nonalcoholic fatty liver disease (NAFLD) is important for prognosis and selection of patients candidates for therapeutic interventions. The reference standard for detecting liver fibrosis is liver biopsy; however, such an invasive procedure, it can be painful and hazardous, and assessment subjective and prone to sampling error. Recently, the serum Enhanced Liver Fibrosis (ELF) Test has been developed for staging liver fibrosis in patients with chronic liver diseases. The aim of our study was to evaluate the ELF Test performance in predicting fibrosis stage in an independent adult cohort of NAFLD patients.
Methods: 82 patients (mean age 46 years) with suspected NAFLD were enrolled undergoing percutaneous liver biopsy and serum sampling. Fibrosis was assessed and scored by using the modified Brunt classification (F0 = no fibrosis; F1 = perisinusoidal/periportal; F2 = perisinusoidal and portal/periportal; F3 = bridging fibrosis; F4 = cirrhosis). The ELF test was determined in all patients by means of an algorithm combining hyaluronic acid, amino-terminal propeptide of type III collagen and tissue inhibitor of metalloproteinase 1. Diagnostic accuracy was assessed determining the area under receiver operating characteristic curves (AUCs).
Results: The distribution of fibrosis stages in our cohort was as follows: F0 = 7.3% (n = 6), F1 = 39.0% (n = 32), F2 = 35.4% (n = 29), F3 = 6.1% (n = 5), F4 = 12.2% (n = 10). The ELF Test had an AUC of 0.988 (95% confidence interval [CI] 0.967–1.008; P < 0.001) for distinguishing cirrhosis, 0.948 (CI 0.883–1.014; P < 0.001) for severe fibrosis, 0.682 (CI 0.568–0.797; P = 0.005) for significant fibrosis and 0.658 (CI 0.401–0.915; P = 0.200) for any fibrosis. ELF scores were significantly higher in patients with severe fibrosis/cirrhosis in respect to ones with no/mild/moderate fibrosis (median 11.26 *vs.* 8.53; P < 0.001). Severe fibrosis and cirrhosis were correctly identified in 91% of patients.
Conclusions: In our cohort of NAFLD patients, the ELF test was able to discriminate severe fibrosis and cirrhosis with an excellent diagnostic accuracy. It may result useful for the selection of cases with more advanced fibrosis stage and for therapeutic follow-up, thus avoiding liver biopsy.

P1036

OBESITY, T2DM, METABOLIC SYNDROME INFLUENCE MORTALITY IN NAFLD IN A COHORT OF MEXICAN PATIENTS

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Background and Aims: The spectrum of non alcoholic fatty liver disease (NAFLD) comprises non alcoholic fatty liver (NAFL), non alcoholic steatohepatitis (NASH) and cirrhosis. Recently, it has been positioning as one of the most frequent causes of chronic liver disease (CLD). Mexico has the second highest worldwide prevalence of obesity and type 2 diabetes mellitus (T2DM) is present in 14% of general population. The aim of this study was to evaluate the influence that metabolic factors had in the natural history of NAFLD in Mexican patients.
Methods: NAFLD diagnosis was made in patients who drank <30 g/day on males, <20 g/day on females. Other etiologies of CLD were ruled out. Demographic, anthropometric, biochemical, US data and comorbidities were registered. All patients had liver biopsy and/or FibroMax. Group 1 (G1): 41 NAFL patients and group 2 (G2): 80 NASH patients. Comorbidities were compared between admission and last visit. Kaplan–Meier curves (KM) were calculated evaluating the development of cirrhosis, its complications and mortality or liver transplant (LT).
Results: During follow-up obesity decreased in both groups, however HBP increased significantly in G1 as T2DM, HBP, dyslipidemia and metabolic syndrome (MS) increased in G2 (Table). Forty patients had cirrhosis initially, and in G2 four patients developed it. Cirrhosis was diagnosed in 44/121 (36%) patients. Twelve out of 121 (10%) patients died/LT, 11 of them were cirrhotic. Causes of death: liver related 9/12 (75%), cardiovascular causes 2/12 (17%) and infection 1/12 (8%). KM curves demonstrated that patients with obesity (p = 0.041), T2DM (p = 0.002) and metabolic syndrome (p = 0.001) had higher mortality/LT. The development of cirrhosis during follow-up was not influenced by comorbidities.

Table: Progression of comorbidities in NAFL and NASH

Comorbidity	Group 1 (n = 41)			Group 2 (n = 80)		
	Admission	Last visit	*P*	Admission	Last visit	*P*
Obesity	18	15	0.687	45	40	0.210
T2DM	11	14	0.250	34	48	<0.001
HBP	8	14	0.031	31	38	0.016
Dyslipidemia	32	35	0.250	56	66	0.002
MS	11	14	0.250	42	46	<0.001

Conclusions: Patients with NASH developed more comorbidities than patients with NAFL in the long term follow-up. Patients with obesity, T2DM and MS had higher mortality/LT. However the development of cirrhosis during follow-up was not influenced by comorbidities. The most common cause of death in NAFLD was liver related.

P1037

INFLUENCE OF PREDICTOR VARIABLES ON FIBROMAX RESULTS

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Background and Aims: Among the noninvasive alternatives to liver biopsy in patients at risk of chronic liver diseases, several studies have demonstrated the predictive value of FibroMax (*BioPredictive, France*). FibroMax includes 5 tests: FibroTest (quantitative assessment of fibrosis), ActiTest (quantitative assessment of necroinflammatory activity in chronic viral hepatitis C and B), SteatoTest (quantitative assessment of steatosis), NashTest (categorical diagnosis of nonalcoholic steatohepatitis) and AshTest (quantitative assessment of alcoholic steatohepatitis). The aim of the study is evaluation of predictor variables influence of Fibromax results.
Methods: 80 patients were involved in the study (86% male), 21–68 years old (42.4±10.2). Statistic analysis was done by SPSS 11.0. Multiple regression was used for estimation of independent or predictor variables (gender, age, BMI, fasting glucose, triglycerides, cholesterol, AST, ALT and GGT) influence on

five Fibromax tests. Predictor variables were coding as dichotomous or categorical. Gender (male, female), fasting glucose, triglycerides, cholesterol, AST, ALT and GGT (normal, abnormal) are considered as dichotomous. According to BMI patients were divided: <25, 25–30 and >30. By age, patients were subdivided into the following groups: <30, 31–40, 41–50 and >50 years old. By fibrosis degree patients were subdivided into 3 groups: 1: F from 0 to 2; 2: F2,3; 3: F3,4. According to necroinflammation activity subdivision was done into the following groups: 1: A from 0 to 1; 2: A 1–2; 3: A 3. By steatosis degree separation: 1: S from 0 to 1; 2: S1–2; 3: S2–3. Nash- and AstTests divide correspondingly on 1, 2 and 3.

Results: According to ANOVA results models for all 5 tests are significant. By using all the predictors simultaneously: for FibroTest 26% (adjusted $R^2 = 0.261$) of the variance can be predicted from the independent variables with significance of age and cholesterol ($p = 0.001$, $p = 0.08$); for ActiTest – 75% (adjusted $R^2 = 0.746$) with significance of ALT, AST, GGT ($p < 0.001$); for SteatoTest – 51% (adjusted $R^2 = 0.509$) with significance of BMI, TG, GGT ($p < 0.001$, $p = 0.002$, $p < 0.001$); for NashTest – 23% (adjusted $R^2 = 0.233$) with significance of gender, BMI, GGT ($p = 0.058$, $p = 0.019$, $p = 0.053$); for AshTest – 13% (adjusted $R^2 = 0.133$) with significance of gender, AST ($p = 0.007$, $p = 0.057$).

Conclusions: Multiple regression has shown that using of all predictor variables simultaneously for prediction of all five Fibromax tests results are significant with predisposing role of particular predictors for different tests.

P1038

NON-ALCOHOLIC FATTY LIVER DISEASE IS A COMMON FINDING IN ASYMPTOMATIC, UNCOMPLICATED TYPE 2 DIABETES MELLITUS PATIENTS ASSESSED BY MULTIPARAMETRIC MAGNETIC RESONANCE LIVER IMAGING

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is considered to be prevalent among patients with long standing type 2 diabetes mellitus (T2DM). Compared with non-diabetics, T2DM patients are at higher risk of more advanced forms of NAFLD, such as steatohepatitis (NASH) and fibrosis. Magnetic resonance techniques are increasingly being used in the assessment of liver disease and have been shown to have a high degree of sensitivity and specificity for diagnosis and staging in a histology comparison study.

We used a multiparametric MR protocol for detailed assessment of liver disease in asymptomatic patients with uncomplicated T2DM and relatively short disease duration.

Methods: 32 patients with T2DM with median diabetes duration of 6.5 [IQR 1.5–8] years and 15 age matching non-diabetic controls (56±1, vs 52±3 years respectively, $p = 0.210$) were recruited. T2DM patients were taking only oral antidiabetic therapies. All participants had a multi-parametric MRI scan including T1 mapping and T2* mapping (for iron quantification) which were used to calculate the liver inflammation and fibrosis (LIF) score (0–4 continuous scale; 0 no disease, 4 severe disease) measuring liver extracellular fluid (reflecting fibrosis and inflammation) and ^{1}H MR spectroscopy (^{1}H MRS) for liver lipid quantification. All scans were analysed by a blinded operator.

Results: The mean LIF score was significantly elevated in T2DM patients (1.95±0.17 vs 0.87±0.1, $p < 0.001$) compared to healthy volunteers. Mean liver fat content for T2DM was 12±1.3% compared to 3±0.7% in normal controls ($p < 0.001$). The mean hepatic iron content assessed by T2* mapping was not different between diabetics and the controls (1.34±0.05 vs 1.3±0.03 respectively, $p = 0.493$). There was a strong correlation between the degree of steatosis measured by ^{1}H MRS and LIF scores ($r = 0.775$, $p < 0.001$). 8 out of total 32 patients with T2DM (25%) had elevated plasma alanine aminotransferase (>40 unit/L), and 16 T2DM patients (50%) had LIF score >2.

Conclusions: Significant NAFLD defined as LIF >2 is present in asymptomatic T2DM patients with minor or no ALT elevations. A multi-parametric MRI liver protocol allows a detailed non-invasive tissue characterisation of liver parenchyma, and identification and quantification of fibrosis, steatosis and iron content. This method promises to answer to a pressing need for a reliable, quick, non-invasive screening, staging and monitoring tool for diabetic liver disease.

P1039

A REVIEW OF OUTCOMES OF LIVER BIOPSIES IN HIV-INFECTED PATIENTS WITHOUT HEPATITIS CO-INFECTION 2002–2012

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Background and Aims: Liver disease is an important cause of morbidity and mortality in HIV infected patients in the presence and absence of chronic viral hepatitis. Liver biopsy is the gold standard for the investigation of these diseases and with increasing use of non-invasive measures of liver disease we performed a review of liver biopsy results at our institution in HIV infected patients without chronic viral hepatitis

Methods: We examined all HIV infected patients without evidence of chronic viral hepatitis co-infection who underwent liver biopsy at the Royal Free Hospital between January 2002 and December 2012. Basic demographic information, indications for biopsy and biopsy results were recorded

Results: *Demographics:* During the study period 143 liver biopsies were performed on 126 individuals from a total HIV positive cohort of 4456 patients (2.8%). All individuals had negative serology for HCV and HBV prior to biopsy. 94 patients (74.6%) were male with a mean age of 49.6 years at biopsy. The mean time between HIV diagnosis and biopsy was 8.1 years (range 0.1 years to 29 years). 18% of patients were asymptomatic from their HIV at the time of biopsy, 43% were symptomatic and 37% had a diagnosis of AIDS ($n = 126$). Indications for biopsy: 69% of biopsies were performed for deranged liver function tests; 12% for evaluation of a mass or potential neoplasm; 5% for assessment of presumed cirrhosis and 4% for presumed NAFLD ($n = 143$).

Biopsy results: Steatosis was noted in 45.4% of biopsy samples with 18.8% showing moderate/severe changes. Fibrosis was noted in 18.8% of biopsy samples with 2.8% being cirrhotic ($n = 143$).

Diagnoses made on liver biopsy: Granulomas were present in 10 biopsies (7%); cholestasis was present in 4 biopsies (2.8%); lymphoma was diagnosed in 8 biopsies (5.6%); definite drug reactions in 6 cases (4.2%); tuberculosis in 8 biopsies (2.8%) and autoimmune hepatitis in 3 patients (2.1%) ($n = 143$).

Conclusions: We report a 10 year retrospective analysis of biopsies in HIV positive/hepatitis negative patients. A large proportion of biopsies demonstrate evidence of steatosis with nearly 20% showing moderate or severe steatosis. This correlates with reported elevated prevalence of fatty liver disease in HIV positive individuals. Notably diagnoses other than steatosis were identified in 25.9% of biopsy specimens suggesting an ongoing role for the use of biopsy as compared to non-invasive tests. Further work will be required to define the HIV population in which liver biopsy is most useful diagnostically.

P1040

SHORT-TERM LOW-DOSE THIAZOLIDINEDIONES CAN BE A USEFUL BRIDGING THERAPY IN NASH PATIENTS WITHOUT SIDE EFFECTS

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Background and Aims: Thiazolidinediones (TZDs) improve insulin resistance and have shown effect in the treatment of NASH. However, only low-dose TZDs are available in non-diabetic patients in South Korea due to national health insurance reimbursement policy. Meanwhile, administration of low-dose TZDs can be used more safely while TZDs have adverse effects such as edema, fatigue, and weight gain. We assessed the effect of low-dose TZDs in non-diabetic NASH patients.
Methods: We conducted a prospective study of biopsy proven NASH patients without diabetes from 2003 through 2013. Patients were treated with low-dose TZDs (rosiglitazone 4 mg or pioglitazone 15 mg, once daily) for 12 weeks. AST, ALT, ALP, total protein, albumin, total cholesterol, triglyceride, fasting blood glucose (FBS), body mass index, and HOMA-IR were measured at baseline and after 12 weeks. Data were compared with Wilcoxon Signed Rank Test.
Results: Twenty-eight patients were enrolled. Ten cases were female with a mean age of 36.3±13.7 years. After 12 weeks, a significant decrease in AST (71.1±42.8 to 37.3±16.9, $p < 0.01$), ALT (121.7±60.1 to 59.8±34.1, $p < 0.01$), FBS (104.2±28.1 to 97.9±15.3, $p = 0.013$) and HOMA-IR (2.3±1.5 to 1.9±1.1, $p = 0.039$) levels was observed without weight gain or other side effects. TZDs did not affect plasma ALP, total protein, albumin, total cholesterol, and triglyceride.
Conclusions: Twelve weeks administration of low-dose TZDs in non-diabetic NASH patients showed beneficial effects on liver function without side effects. Low-dose TZDs can be used as a useful bridging therapy while weight loss by dietary control and exercise takes time to be effective.

P1041

RAPID IMPROVEMENT OF HEPATIC STEATOSIS AS ASSESSED BY CONTROLLED ATTENUATION PARAMETER AFTER A TWO WEEK PROTEIN-ENRICHED LOW-CALORIE DIET (HEPAFAST)

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) has become one of the most prevalent liver diseases. NAFLD increases the risk of fibrosis and cirrhosis and is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma. Previous intervention studies have been hampered by the need for liver biopsies and the lack of alternative inexpensive methods for the quantitative measurement of liver fat contents. Hence the primary aim of this study was to avail of the controlled attenuation parameter as quantitative tool for the assessment of therapeutic effects of a two-week low-calorie diet.
Methods: In this prospective single center study, 59 patients with NAFLD received a 14-day low-calorie liver specific diet (HEPAFAST) containing 1000 kcal per day (41% protein, 29% carbohydrate, 24% fat and 6% fiber). The following parameters were assessed at baseline and after 14 days: hepatic fat contents using controlled attenuation parameter (CAP) during transient elastography (FibroScan); body composition with bioimpedance analysis; and serum liver function tests and lipid profiles using standard clinical-chemical assays.
Results: All 59 patients (median age 55 years, range 25–78; 51% women; median BMI 31.7 kg/m^2, range 22.4–43.5) successfully completed the study. A significant reduction in hepatic steatosis (14.1%; $P < 0.0001$) was observed after only 2 weeks: The median CAP score decreased from 293 dB/m (range 177–400) at baseline to 263 dB/m (100–353). In parallel, BMI decreased significantly ($P < 0.0001$), and 30.5% could be reclassified into a lower BMI category. Moreover, body and visceral fat contents were significantly ($P < 0.0001$) reduced by 7%. Serum triglyceride, total LDL, and the LDL/HDL index as well as g-GT activities also decreased significantly (all $P < 0.001$). Interestingly, 11 patients (72% women) demonstrated a CAP increase by 3% after the 2-week intervention despite improvements in body composition, thus were classified as hepatic non-responders. In contrast, a subgroup analysis of the responders revealed a decrease of 16% in median CAP scores from 308 to 261 dB/m.
Conclusions: This elastography-based non-invasive study shows, for the first time, improvements in hepatic steatosis, as quantified non-invasively by CAP, after a short-term protein-enriched low-calorie diet. The dietary intervention not only reduced body weight but improved both body and liver compositon in NAFLD. Compartment- and sex-specific HEPAFAST effects should be investigated further.

P1042

DEREGULATION OF DE NOVO LIPOGENESIS CAN BE ASSOCIATED WITH LIVER DAMAGE IN PATIENTS WITH NON ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Adipose tissue insulin resistance (IR) and elevated adipose free fatty acid (FFA) flux are prominent features in NAFLD. De novo lipogenesis (DNL) is up to 3-fold higher in subjects with fatty liver and is not suppressed on fasting. When DNL is stimulated, the production of saturated FAs is increased and the oxidation of FAs of any source is reduced. Both mechanisms can favor inflammation and IR. We measured FFA flux/composition and used a surrogate index of DNL (DNLi) to evaluate their relationship with histological features in a group of non-diabetic subjects with biopsy-proven NAFLD.
Methods: Hepatic and adipose tissue-IR indices were derived from [$^2H_5$]glycerol and [$^2H_2$] glucose kinetics in a group of non-diabetic NAFLD patients in the basal state ($n = 40$) and after a 4 h oral glucose load test ($n = 20$). Gas chromatography mass spectrometry was used to assess FFAs composition. DNLi was derived as the ratio palmitic/linoleic acid.
Results: Fasting plasma glucose/insulin, lipid profile, hepatic/adipose tissue IR indices and DNLi were similar in the two groups. Fasting DNLi was associated with triglycerides (TG) and FFAs levels and with adipose tissue IR ($r = 0.597$, $r = 0.330$ and $r = 0.394$, respectively). Among histological features, fasting DNLi significantly correlated with steatosis ($r = 0.364$, $P = 0.02$) and NAS score ($r = 0.306$, $P = 0.05$). After the glucose load, TG levels initially increased despite elevated insulin levels, suggesting a significant contribution of DNL. Accordingly, DNLi consensually increased with TG levels ($r = 0.749$, $P < 0.005$) and was significantly related to the degree of fibrosis (r_s=0.514, $P = 0.02$).
Conclusions: Oral glucose load is associated with changes in DNL and hepatic triglyceride synthesis that can favor liver fibrosis in patients with NAFLD.
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P1043

EXERCISE REDUCES LIVER FAT, VISCERAL FAT AND CIRCULATING TRIGLYCERIDES, BUT NOT CIRCULATING INFLAMMATORY MARKERS IN NON-ALCOHOLIC STEATOHEPATITIS

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Background and Aims: Non-alcoholic steatohepatitis (NASH) represents steatosis and ballooning degeneration, inflammation and fibrosis. Current treatments for NASH are limited, with no studies to date reporting the effects of exercise in people with NASH. Studies in NAFLD show promising effects on liver lipid but the effect on inflammation and visceral fat, key mediators of fibrosis, are not known. *Aims:* Determine the effect of a 12-week exercise intervention on liver lipid and circulatory inflammation in people with NASH.

Methods: 24 participants (mean age 52±14 years, BMI 33±6) with histologically characterised NASH (NAFLD Activity Score ≥5) received either: resistance exercise (n = 12) or continue standard care (n = 12) over 12 weeks and maintained baseline weight. Participants were not undergoing insulin sensitising treatment, dietary change or regular activity. Subjects with heart/kidney disease or in vivo ferrous material were excluded. Liver lipid content, subcutaneous and visceral adiposity were assessed using magnetic resonance techniques, inflammation, fibrosis markers, insulin sensitivity were assessed at baseline and at 12 weeks.

Results: Resistance exercise produced a significant reduction in liver lipid content (−13±24 vs. 6±15%, P < 0.05), visceral fat (1876±530 vs. 1698±500 cm^2, P < 0.05) and plasma triglycerides (2.2±1.0 vs. 1.7±0.8 mmol/L, P < 0.05). There was no effect of exercise on circulatory inflammation (IL6: 1.4±0.8 pg/mL to 1.7±1.7 pg/mL vs. 2.4±4.6 pg/mL to 2.1±2.9 pg/mL; TNFα: 2.2±0.6 pg/mL to 2.4±0.8 pg/mL vs. 2.3±0.7 pg/mL to 2.3±0.8 pg/mL, P > 0.05) or abdominal adiposity (VAT: 187±54 cm^2 to 170±50 cm^2 vs. 170±72 cm^2 to 211±112 cm^2; SAT: 337±181 cm^2 to 317±158 cm^2 vs. 389±121 cm^2 to 394±112 cm^2, P > 0.05). Metabolically, there was no effect of exercise on HbA1c (51±14 mmol/mol to 49±12 mmol/mol vs. 46±11 mmol/mol to 49±12 mmol/mol, P > 0.05), HOMA IR (2.3±1.4 to 1.9±0.8 vs. 1.9±1.1 to 1.7±1.1, P > 0.05) or 2hour glucose levels or liver enzymes (ALT: 53±25 U/L to 52±18 U/L vs. 81±59 U/L to 71±52 U/L; AST: 41±14 U/L to 45±12 U/L vs. 59±41 U/L to 58±45 U/L, P > 0.05).

Conclusions: This is the first study reporting the effects of exercise on liver lipid, body composition and circulating inflammation in patients with histologically defined NASH. Exercise produces a significant reduction in liver lipid, visceral fat and plasma triglycerides but no effect on body weight, abdominal adiposity, or circulatory markers of inflammation. These results suggest that exercise alone may be insufficient to target the mediators of NASH and warrants further exploration.

P1044

RANDOMISED, PLACEBO-CONTROLLED CLINICAL TRIAL: LONG-TERM RESVERATROL TREATMENT FOR NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: 'The obesity epidemic' has led to an increase in obesity-related conditions including non-alcoholic fatty liver disease (NAFLD), for which effective treatments are in demand. The polyphenol resveratrol (RSV) prevents the development of experimental NAFLD in numerous *in vitro* and *in vivo* studies through anti-inflammatory, anti-oxidant and AMPK/SIRT1 activating effects. We hypothesized that RSV would alleviate NAFLD in a randomized, placebo-controlled, double-blind, clinical trial.

Methods: 28 overweight, non-diabetic patients with transaminasemia and histological NAFLD were recruited for the trial and randomized to placebo or RSV 500 mg t.i.d. for 6 months. 26 patients completed the trial and underwent repeated clinical investigation, blood work and MR spectroscopy. 19 patients also committed to a repeat liver biopsy.

Results: There was a decrease in liver steatosis as assessed by MR spectroscopy in RSV treated patients from 31% to 23% (P = 0.03), in placebo treated patients from 32 to 30%, however there was no statistical difference between the two treatment arms (P = 0.38). RSV treatment was no better than placebo to improve histological NAFLD activity score (P = 0.59), steatosis (P = 0.22) or fibrosis (P = 0.75). We detected no difference in ALT (P = 0.51) or in the level of other plasma markers of liver injury when comparing the RSV and placebo groups. Similarly, we observed no improvements in markers of the metabolic syndrome (central obesity, plasma lipids, glucose tolerance, blood pressure).

Conclusions: In contrast to findings in experimental NAFLD, high-dose, long-term RSV treatment had no consistent therapeutic effect in alleviating clinical or histological NAFLD.

P1045

ULTRA-HIGH-FIELD MR-SPECTROSCOPY IN NAFLD AS NON-INVASIVE IN-VIVO TOOL FOR MONITORING CHANGES IN FAT AND ENERGY METABOLISM WITH POTENTIAL IDENTIFICATION OF NASH AND ADVANCED FIBROSIS BY SATURATION TRANSFER TECHNIQUE

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Background and Aims: With the rising incidence of non-alcoholic fatty liver disease (NAFLD) with potentially progressive steatohepatitis (NASH) non-invasive tools for risk stratification and treatment response assessment are urgently needed. We therefore used ultra-high-field magnetic-resonance-spectroscopy (MRS) as non-invasive tool to obtain novel mechanistic and pathogenetic insights into alterations of hepatic metabolism in NAFLD including ATP flux measured by saturation transfer technique.

Methods: MRS and liver biopsy were performed back-to-back in suspected NAFLD patients and data were correlated with histology. Hepatocellular lipid content (HCL) was measured by ^{1}H-MRS. 7.0-T ^{31}P-MRS was applied to determine phosphomonoester (PME), phosphodiester (PDE), phosphocreatine (PCr), ATP as well as total phosphate (TP). Additionally, saturation-transfer was measured to get a more dynamic in-vivo insight in energy metabolism.

Results: 30 patients (12 female) were included. Median age was 52 years (24–70). Histological diagnosis was simple steatosis (SS; n = 8) and NASH (n = 22).

Steatosis assessed by ^{1}H-MRS correlated well with histology (r = 0.704; p < 0.001). All NASH patients showed significantly higher values of steatosis compared to SS (r = 0.69, p < 0.001). PME, such as PE/TP ratio as marker of cell membrane alterations increased from low fibrosis (LF; 0–2) to advanced fibrosis (AF; 3+4) (r = 0.556; p = 0.002) assessed by ^{31}P-MRS. Conversely, GPC/PME+PDE decreased (r = −0.531, p = 0.004) and PCr/TP increased

(r = 0.458, p = 0.014) in cirrhosis and AF, respectively. γ-ATP as most robust marker of ATP was significantly lower in AF than LF (r = −0.376; p = 0.032), whereas the mean exchange rate constant (r = −0.485; p = 0.012) and the ATP flux (r = −0.431; p = 0.025) were lower in NASH than SS patients showing a dynamic energy change for NASH.

Conclusions: High field ^{1}H-MRS strongly correlates with histological grades of steatosis showing also differences between SS and NASH. In-vivo ^{31}P-MRS shows promising results indicating changes in hepatic cell membrane and energy metabolism in inflammation and fibrosis associated with NASH and AF. Non-invasive profiling in NAFLD appears feasible and may serve to assess therapeutic efficacy of new treatment approaches.

P1046

A ONE YEAR RETROSPECTIVE REVIEW OF NEW PATIENT ATTENDANCES AT A TERTIARY HEPATOLOGY CENTRE HIGHLIGHTING THE INCREASING CHALLENGE OF NAFLD AND THE NEED TO DEVELOP CLINICAL PATHWAYS

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Background and Aims: 25% of the UK population is overweight or obese. 75% will have NAFLD of whom 5% will have steatohepatitis and associated fibrosis. Identifying this subgroup at risk of chronic liver disease can be challenging as they are often asymptomatic. Non-invasive tests (NIT) of liver fibrosis including FIB4 and the Enhanced Liver Fibrosis (ELF) test have been validated in NAFLD and provide the opportunity to design pathways to stratify patient disease severity in primary care.

We performed a retrospective review of GP referrals to secondary care of patients with a final diagnosis of NAFLD. This will be followed by a prospective evaluation of a novel Camden and Islington CCG commisioned pathway using non-invasive tests to stratify liver fibrosis in patients with NAFLD.

Methods: All new GP referrals to the Royal Free London NHS Foundation Trust with a final diagnosis of NAFLD between 1/4/12 and 31/3/13 had their electronic patient records and patient notes reviewed. Demographic, clinical, laboratory and outcome data were recorded. The potential impact of the use of FIB4 was assessed. Prospectively FIB4 and ELF are being used to stratify patients prior to referral.

Results: In the retrospective analysis 112 patients with a final diagnosis of NAFLD were referred by GP's over 1 year. 74 patients (66%) had FIB4 <1.30 (low risk of advanced liver disease). FIB4 was indeterminate (1.30–3.25) in 36 (31%) who would be stratified with ELF in the new pathway.

Eighteen (16%) had a liver biopsy. Six (5%) had FIB4 <1.30, each of whom had a histological stage of ≤F2 fibrosis and could have avoided referral under the new pathway. Of 6 (5%) with confirmed cirrhosis on biopsy, one had FIB4 >3.25 and 5 had indeterminate FIB4 scores (1.30–3.25).

Conclusions: The NAFLD epidemic mandates new strategies to manage the increasing burden effectively. Those with advanced liver disease need specialist input, whilst those at low risk should have their risk factors aggressively addressed in primary care. Current approaches are ineffective in stratifying patients for referral. Using FIB4 alone, 66% of referrals could have been avoided but the majority of cirrhotic patients would not have been identified. We have implemented a novel primary care pathway integrating the use of FIB4 and ELF (to categorise FIB4 indeterminate cases) to stratify patients for management in primary or secondary care (Figure 1). Evaluation of clinical and cost effectiveness is underway.

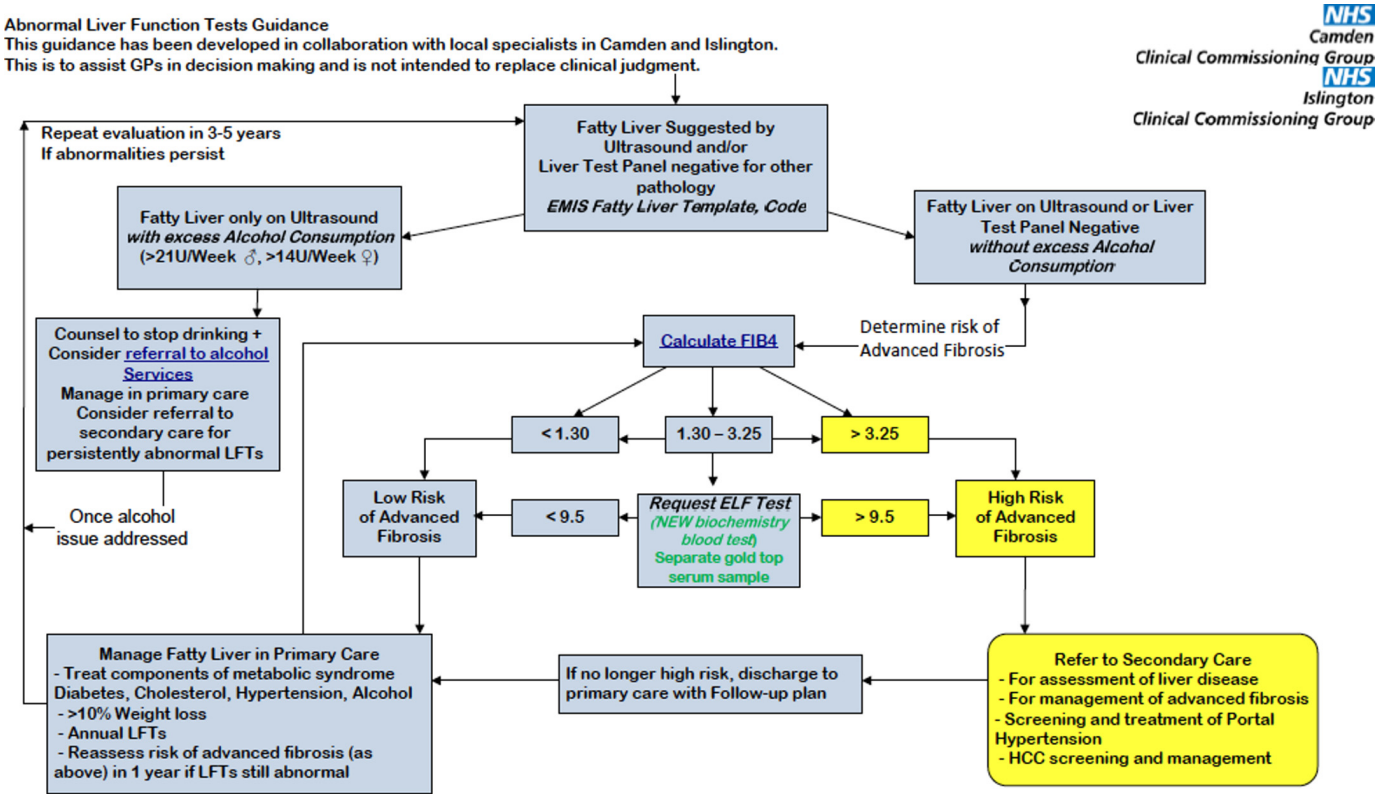

Figure 1 (abstract P1046). Abnormal liver function tests guidance.

P1047

CONTROLLED ATTENUATION PARAMETER (CAP): COMBINATION OF M AND XL PROBE IMPROVES FEASIBILITY AND ACCURACY FOR DISCRIMINATING ADVANCED GRADES OF STEATOSIS

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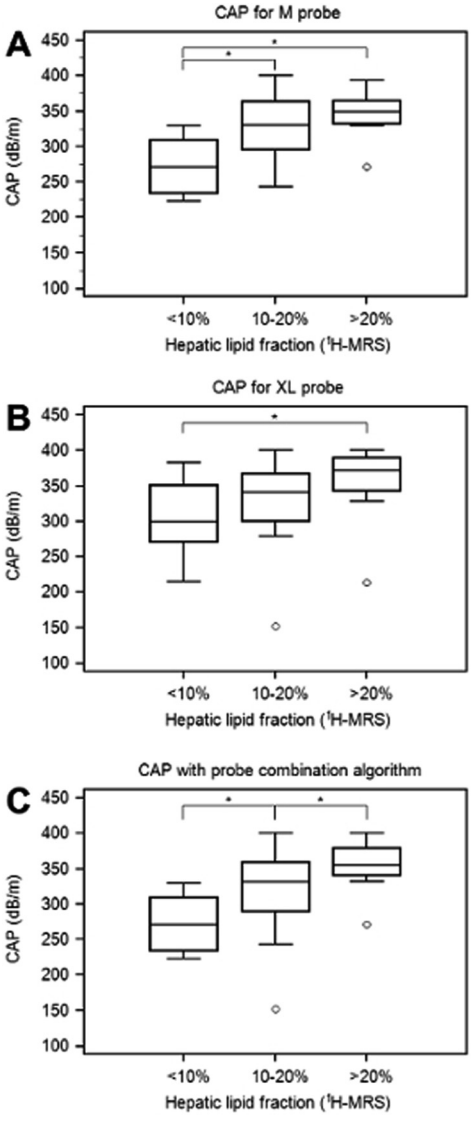
Background and Aims: Non-invasive steatosis quantification is of emerging importance for the characterization of patients with fatty liver disease. The controlled attenuation parameter (CAP) estimates steatosis during the measurement of liver stiffness with Fibroscan®. However, feasibility and accuracy of CAP measurement using the conventional M probe (CAP-M) is limited in obese subjects. We therefore compared applicability and diagnostic accuracy of CAP-M with the new CAP algorithm for the XL probe (CAP-XL), dedicated to obese patients.



Methods: Type 2 diabetic patients (n = 51) at risk for non-alcoholic fatty liver disease were evaluated with both CAP-M and CAP-XL. Skin-to-liver-capsule distance (SLD) at the measuring site was used for probe stratification (CAP-combined): CAP-M in cases with SLD <25 mm, CAP-XL for cases with SLD ≥25 mm. Proton magnetic resonance spectroscopy (^{1}H-MRS, liver segments II and VII) served as reference standard.

Results: 47 patients (49% female; age 65.2+8.5 years; body mass index 29.5+4.8 kg/m^2; 28% with SLD ≥25 mm) had valid ^{1}H-MRS and were included in the final analysis. CAP-M was feasible in 43 (91%), CAP-XL in 46 (98%) and CAP-combined in 46 (98%) cases, respectively. CAP values correlated with ^{1}H-MRS results: r = 0.4473 [0.1698; 0.6591] for CAP-M, r = 0.3238 [0.0369; 0.5613] for CAP-XL and r = 0.4186 [0.1460; 0.6321] for CAP-combined ($p < 0.05$ respectively).

CAP-M and CAP-XL values differed significantly between patients with low (<10% lipid fraction) and high (>20% lipid fraction) hepatic fat content (Figures A and B, *ANOVA $p < 0.05$). Only CAP-combined discriminated between all grades of steatosis (Figure C) and showed good diagnostic accuracy for detection of patients with >10% lipid fraction (n = 35; AUC 0.847, sensitivity 69%, specificity 100%) and >20% lipid fraction (n = 15; AUC 0.788, sensitivity 93%, specificity 68%).

Conclusions: CAP-XL optimizes applicability of non-invasive steatosis quantification, but is imprecise in cases with lower grades of steatosis if used alone. Combination of CAP-M and CAP-XL increases the accuracy for discriminating advanced stages of steatosis.

P1048

EFFECTS OF PROSTEATOGENIC TM6SF2 AND NCAN/SUGP1 VARIANTS ON HEPATIC STEATOSIS AND NON-INVASIVE MARKERS OF LIVER INJURY IN PATIENTS WITH CHRONIC LIVER DISEASES

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Background and Aims: Previously we demonstrated that the frequent genetic variant *PNPLA3* p.I148M is associated with increased hepatic steatosis quantified by controlled attenuation parameter (CAP) and fibrosis measured by liver stiffness (LS) in patients with chronic liver diseases (CLD). Recently single nucleotide variants in the *TM6SF2* gene (rs58542926) and the *NCAN/SUGP1* locus (rs10401969) have been reported as additional genetic determinants of fatty liver (Liu et al. *Nat Comm* 2014; DiStefano et al. *Acta Diabetol* 2014). Here, we investigate the associations between all of these variants and surrogate markers or liver injury, including CAP and LS, in patients with CLD.

Methods: In total, 174 patients with non-viral and non-cholestatic CLD (50% men, age 18–77 years) were recruited for the study. Hepatic steatosis was phenotyped by CAP during transient elastography (FibroScan). Genotyping was performed using Taqman assays. Statistical analyses included exact tests of Hardy–Weinberg equilibrium (HWE), association tests in contingency tables as well as non-parametric tests for continuous variables.

Results: Overall, the median CAP score was 285 dB/m (100–398), indicating moderate and severe steatosis in the majority of patients, but median LS was 6.1 kPa only (1.6–69.1). CAP values correlated with ALT activities (r = 0.236, P < 0.01) and LS (r = 0.174, P < 0.05). We observed the following allele frequencies, all of which were in HWE (P > 0.05): *TM6SF2* [EE] 139 (79.9%), [EK] 33 (19.0%), [KK] 2 (1.1%); *SUGP1* [CC] 137 (78.7%), [CT] 34 (19.5%), [TT] 3 (1.7%). Carriers of the *TM6SF2* and *SUGP1* risk alleles presented with significantly (all P < 0.05) increased serum ALT [57 U/l (26–168) *vs.* 44 U/l (9–2106)

and 58 U/l (26–168) *vs*. 44 U/l (9–2106), respectively]. In contrast to the common *PNPLA3* mutation p.I148M, neither *TM6SF2* nor *SUGP1* genotypes were significantly associated with higher CAP or LS, or with an increased risk of presenting CAP ≥238 dB/m, which is characteristic for liver steatosis (all P > 0.05).
Conclusions: Our study indicates that the *TM6SF2* and *NCAN/SUGP1* variants do not display marked effects on hepatic steatosis, but carriers of the risk alleles present with increased liver inflammation. Given the low frequencies of the *TM6SF2* risk genotypes in the general population, variant *PNPLA3* remains the clinically most relevant prosteato- and profibrogenic genetic factor.

P1049
LYSOSOMAL ACID LIPASE ACTIVITY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is considered the hepatic component of the metabolic syndrome. Nevertheless, some genetic factors influence the onset and progression of NAFLD. Lysosomal Acid Lipase (LAL) is a key enzyme in the lipid metabolism, and LAL deficiency causes accumulation of lipids and pre-mature atherosclerosis. There are no data on factors influencing LAL activity in patients with NAFLD.
Methods: NAFLD was diagnosed by ultrasonographic Hamaguchi's criteria in 200 subjects with no history of alcohol abuse, negative tests for HCV and HBV and for autoimmune liver disease. LAL activity was measured with dried blood spot using Lalistat 2, an inhibitor of LAL.
Results: Mean age was 52.2±11.0 years and 58% were male. In the whole cohort the median LAL activity was 0.77 [0.59–0.99] nmol/spot/h. We found a slightly reduced LAL activity in men than women (0.75 [0.57–0.95] vs. 0.82 [0.65–1.07], p = 0.079), and no correlation with age or Hamaguchi score. We found a significant difference between the lowest and the highest tertile of LAL activity for total cholesterol (206.8±39.7 mg/dl vs 192.9±34.7 mg/dl, p < 0.05), LDL-c (124.1±30.3 mg/dl vs 111.8±31.5 mg/dl, p < 0.05), AST (25 [19.0–34.5] UI/L vs. 21.0 [17.0–28.0] UI/L, p = 0.032), ALT (33.0 [23.0–52.5] UI/L vs. 28.0 [20.0–38.7] UI/L, p = 0.027), platelets (221.6±53.6 vs. 258.5±67.3 $\times 10^3/\mu l$, p = 0.001) and APRI index (0.31 [0.24–0.49] vs. 0.25 [0.18–0.31], p = 0.002). Furthermore, patients taking statins had a significant higher LAL activity (0.73 [0.55–0.95 vs. 0.91 [0.66–1.12], p = 0.008). A stepwise multivariable linear regression analysis showed that platelets (B: 0.540, p < 0.001), statin use (B: 0.185, p = 0.009), AST (B: −0.634, p = 0.006), APRI index (B: −0.573, p = 0.024) were independently associated with LAL activity.
Conclusions: This is the first report on LAL activity in NAFLD patients. LAL activity was inversely correlated with AST and APRI index and directly with platelet count. Statins use was positively associated with LAL activity. Understanding the role of LAL in NAFLD may provide novel insights in the pathogenesis of NA

P1050
ASSOCIATION OF FAMILY HISTORY OF METABOLIC TRAITS WITH AGE AT DIAGNOSIS AND SEVERITY OF NON ALCOHOLIC STEATOHEPATITIS (NASH) RELATED CIRRHOSIS

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Background and Aims: Familial aggregation of metabolic traits (MTs) such as diabetes, hypertension, dyslipidemia, coronary artery disease and obesity with fatty liver disease, non alcoholic steatohepatitis (NASH) and advanced fibrosis is well documented. However, there is scarcity of data regarding such association with NASH related cirrhosis. We aimed to explore the association of positive family history (FH) of MTs with age at diagnosis, severity and complications of NASH related cirrhosis.
Methods: A cross-sectional study was conducted at a tertiary care centre and all in and out patients of cirrhosis with underlying aetiology as NASH were included during the period 2013–14. Other common etiologies (like ALD, HBC, HCV autoimmune etc) of cirrhosis were excluded. FH, demographic characteristics, medical history data, anthropometrical measurements and laboratory data were recorded. Positive FH for MTs was decided if at least one first degree relative had a history of any of these MTs.
Results: A total of 504 cirrhotics [age 53.1±12.3 yrs, male 392 (77.8%), female 112 (22.2%)] were enrolled. Positive FH for MTs was seen in 328 (65.1%) cases. Both parents had either DM or/and HTN in 159 (31.6%) and in one of the parents in 162 (32.2%) cases. History of 1–2, 2–5 or >5 MTs among parents was seen in 117 (23.3%), 148 (29.5%) and 12 (2.4%) cases, respectively. Sixty (11.9%) cases had a history of cirrhosis among their parents. Cirrhotics with positive FH had significantly lower age at diagnosis (48.1±10.7 vs 52.1±11.7 yrs), high BMI (28.5±5.3 vs 26.4±4.3 kg/m^2), high MELD (10.6±6.9 vs 9.3±3.4), high CTP (8.1±2.5 vs 6.9±2.1) and had more complications like ascites (46.6% vs 20.5%), variceal bleed (25.9% vs 14.2%), hepatic encephalopathy (25.9% vs 6.8%) and jaundice (15.5% vs 8.0%), (p < 0.05). Age, gender and BMI adjusted odds ratios were calculated. FH of MTs increased the risk of getting cirrhosis at an early age of <45 yrs (OR: 4.9, 95% CI 2.3–10.4), with MELD >15 (OR: 1.6, 95% CI 1.1–2.3), with Child C (OR: 3.2, 95% CI 1.6–6.1) and with one or more complications (OR: 4.9, 95% CI 3.2–7.6).
Conclusions: We conclude that a positive FH for MTs leads to an early and severe NASH related cirrhosis with high odds of development of complications. Further studies are needed to better understand the mechanistic pathway behind this association. Health education strategies to disseminate messages regarding severe liver disease among this high risk group with positive FH of MTs, is a prime requisite.

P1051
ALGINATE ENRICHED BREAD ATTENUATES CIRCULATING LIPIDS AND NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: *Background:* Non-alcoholic fatty liver disease (NAFLD) affects up to one in three people of developed nations and is considered by some to be the hepatic manifestation of the metabolic syndrome. People with NAFLD have a high level of fat intake which may cause (1) high energy intake, (2) insulin resistance, and (3) hepatic de novo lipogenesis. Therapeutic interventions for NAFLD are limited. Alginates are

polysaccharides extracted from brown algae that are un-digestible in the upper gastrointestinal tract (GI). Specific alginates are able to inhibit the activity of pancreatic lipase and thus reduce fat digestion and absorption. The effect of alginates based in food upon fat absorption is not known, despite the therapeutic implications. *Aims:* To determine if alginate enriched bread inhibits fat digestion and circulatory lipids.

Methods: Twenty-nine ileostomy patients were fasted overnight and then fed either 100g alginate enriched bread (4% w/w wet dough) with 20g of butter, or 100g of control bread with 20g of butter a month apart in a double blind randomised cross over study. Both alginate and control bread were produced by Greggs Plc master baker. Effluent samples, blood samples, and wellbeing questionnaires were taken at baseline and then every 30 minutes for a total of 5 hours. Ileostomy patients were used to create a model to study fat absorption in the upper GI in isolation.

Results: The alginate bread produced a 20% increase in effluent weight at 300 minutes (2489g vs. 2010g, $P < 0.05$), a 31% increase in total effluent fat (376g vs. 264g, $P < 0.05$) and a significant correlation between fat content effluent weight ($r = 0.98$, $P < 0.05$). A small reduction in plasma triglycerides was reported when consuming alginate bread, however this was not significant ($P > 0.05$). There were no substantial differences in palatability between the two breads, apart from time point zero where subjects reported an increase in thirst (1.5 ± 1.5 vs. 0.5 ± 0.5, $P < 0.05$) and reduced fullness (2.2 ± 1.8 vs. 1.6 ± 1.6, $P < 0.05$) following consumption of alginate bread.

Conclusions: This is the first study to show that alginate enriched products reduce fat digestion in man. The data shows that alginate enriched products are able to attenuate fat digestion by up to 31%. Alginate enriched products hold potential as a therapeutic weight and metabolic management therapy, without side effects of other known pharmaceutical agents, and should be considered in NAFLD.

P1052

AN IMBALANCE BETWEEN Th17 AND Treg KEY TO PATHOGENESIS OF NON-ALCOHOLIC FATTY LIVER DISEASE IN MORBIDLY OBESE PATIENTS

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Background and Aims: An adequate balance between Treg/Th17 is crucial for the stability of immune homeostasis; a change in this balance can lead to autoimmunity, chronic inflammation and the development of liver diseases. Therefore, the aim of our study was to assess a possible imbalance Treg/Th17 in morbidly obese (MO) patients before and after bariatric surgery (BS) and to correlate these results with the progression of NAFLD.

Methods: Prospectively, 49 MO patients undergoing BS and 17 healthy controls (HC) have been enrolled in our study. Peripheral blood (PB) rate of Treg (CD4+CD25highFOXP3+) and Th17 (CD4+CD161+CCR6+IL17+) were analyzed with flow cytometry before and, in 42 MO, 6 months after BS. Liver biopsies obtained during surgery were classified according to Brunt classification, and the rate of intrahepatic Treg (CD4+CD45+CD25highCD127lowFOXP3+) and Th17 were assessed by flow cytometry.

Results: According to liver histology, 29% of patients displayed a variable inflammatory infiltrate (NASH), 41% had simple steatosis (SS), 19% had normal histology (NH) and in 11% liver histology could not be determined. MO patients showed a significant lower rate of PB Treg when compared to HC (2.52 ± 1.45 vs 3.43 ± 1.23; $p = 0.029$), as well as a lower Treg/Th17 ratio (20.53 ± 31.36 vs 52.29 ± 41.92; $p = 0.003$). Regarding liver histology, we found no differences in the percentage of Treg or Th17 in PB. Nevertheless, we observed a higher rate of intrahepatic Treg and Th17 in patients with NASH compared to those with SS (Treg: 2.9 ± 1.81 vs 1.96 ± 0.7) (Th17: 1.49 ± 0.55 vs 1.18 ± 0.47), whereas Treg/Th17 ratio was lower (1.39 ± 0.71 vs 2.14 ± 1.35). Six months after BS, the rate of PB Treg and Th17 significantly increased when compared to baseline levels (Treg: 3.49 ± 1.15 vs 2.75 ± 1.44; $p < 0.001$) (Th17: 0.57 ± 0.8 vs 0.21 ± 0.24; $p = 0.023$). This increment was greater in the case of Treg, as reflected in the increase of Treg/Th17 ratio (24.88 ± 72.91 vs 18.58 ± 29.15; $p = 0.06$).

Conclusions: In the liver of morbidly obese patients with NASH, there is an increase in Treg and Th17 populations but with a decrease in the Treg /Th17 ratio compared to patients without NASH. This imbalance may be key to understand NASH pathogenesis in morbidly obese patients. In addition, we observed a considerable discrepancy between peripheral and intrahepatic Treg/Th17, suggesting that changes in adaptive immunity cells are confined to the liver and therefore, we should not over interpret peripheral blood results.

P1053

PREVALENCE OF PATATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING 3 (PNPLA3) rs738409 C/G POLYMORPHISM IN PATIENTS WITH BONA-FIDE POST-NASH CIRRHOSIS

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Background and Aims: The patatin-like phospholipase domain containing 3 (PNPLA3) rs738409 C/G single nucleotide polymorphism (SNP) has been consistently associated with steatosis and fibrosis in NAFLD populations undergoing liver biopsy, in which cirrhotic patients were very poorly represented. Since not all NAFLD with fibrosis evolve to cirrhosis, we aimed to determine the prevalence of this SNP in a group of patients with post-NASH cirrhosis.

Methods: Consecutive patients with *bona-fide* post-NASH cirrhosis were enrolled. A sample of patients with biopsy-proven non-cirrhotic NAFLD served as controls and results were also compared to published data from a population of Italian blood donors (Valenti L *et al.*, 2010). All other causes of liver disease were excluded and cirrhotic patients were required to have documentation of at least one of the followings preceding the diagnosis of cirrhosis: ultrasonographic steatosis; metabolic syndrome; diabetes mellitus; obesity. The C/G SNP was analyzed by pyrosequencing method in PyroMark_Q96 ID instrument.

Results: Thirty-six patients with *bona-fide* post-NASH cirrhosis and 45 patients with non-cirrhotic NAFLD were studied. Diagnosis of cirrhosis was histological in 13 cases and clinical in 23 cases. Among non-cirrhotic NAFLD cases, 33 were NASH and 12 non-NASH and fibrosis was as follows: 4 (F0); 21 (F1); 9 (F2); 11 (F3). Almost 80% of patients with post-NASH cirrhosis held at least a copy of the G allele and 56% carried the homozygote GG genotype, compared to 56% and 20% in the non-cirrhotic NAFLD group and 34% and <5% in blood donors, respectively (Table 1). Compared to the non-cirrhotic NAFLD group, carriage of the G allele [unadjusted OR 2.8 (1.05–7.47)], and particularly of the GG genotype [unadjusted OR 3.86 (1.50–9.95)], was strongly associated with post-NASH cirrhosis. In a multivariate model including age, sex, BMI, diabetes, and PNPLA3 rs738409 genotype, only age [years, OR 1.05 (1.05–1.19)] and presence of the G allele [OR 2.2 (1.11–4.48)], or better of the GG genotype [OR 4.13 (1.22–14.0)], were significantly associated with cirrhosis.

Genotype	Post-NASH cirrhosis	Noncirrhotic NAFLD	Unadjusted OR	p value	Italian blood donors [a]	Unadjusted OR	P value
CC	8 (22%)	20 (44%)	–	–	118 (66%)	–	–
CG	8 (22%)	16 (36%)	1.25 (0.38–4.07)	0.7	56 (31%)	2.11 (0.75–5.96)	0.16
GG	20 (56%)	9 (20%)	2.36 (1.33–4.16)	0.003	5 (2.8%)	13.5 (5.2–34.9)	<0.001

[a] Valenti L et al., 2010.

Conclusions: Prevalence of the PNPLA3 rs738409 C/G SNP, and particularly of the GG genotype, is extremely high in patients with *bona-fide* post-NASH cirrhosis and significantly higher than that in non-cirrhotic NAFLD patients, suggesting that most of NASH cases progressing till cirrhosis require the contribution of an altered PNPLA3 function.

P1054
LIVER ENZYMES, ADIPONECTIN AND VITAMIN D LEVELS AS PREDICTORS OF DIABETES – DATA FROM THE HEINZ NIXDORF RECALL STUDY

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Background and Aims: Key features of the metabolic syndrome are insulin resistance and diabetes. The liver as central metabolic organ is not only affected by the metabolic syndrome as non-alcoholic fatty liver disease (NAFLD), but may contribute to insulin resistance and metabolic alterations. Thus we aimed to identify potential associations between liver injury markers and diabetes in the population-based Heinz Nixdorf Recall Study.
Methods: Demographic and laboratory data were analyzed in participants (n = 4814, age 45 to 75y). Metabolic profile included body-mass-index (BMI; kg/m^2), type-2-diabetes rate, as well as blood levels of adiponectin, vitamin D, and C-reactive protein (CRP).
Results: ALT and AST values were significantly higher in male subjects than in females. Mean BMI was 27.9 kg/m^2 and type-2-diabetes was present in 656 participants (13.7%). Adiponectin and vitamin D both correlated inversely with BMI. ALT, AST, and GGT correlated with BMI, CRP, and HbA1c and inversely correlated with adiponectin levels. Utilizing machine learning techniques, a computational model was built with a high accuracy for the prediction of diabetes from blood parameters. Moreover, the model identified adiponectin, GGT, and vitamin D levels as highly important for the prediction.
Conclusions: Transaminase levels were closely associated with the BMI and the risk for diabetes, even within normal ranges. Transaminase levels and adiponectin were inversely associated and represent predictors of diabetes in this cohort. Re-assessment of current normal range limits should be considered, to provide a more exact indicator for chronic metabolic liver injury, in particular to reflect the situation in diabetic, overweight or obese individuals.

P1055
THE MACROPHAGE ACTIVATION MARKER SOLUBLE CD163 IS INDEPENDENTLY ASSOCIATED WITH THE SEVERITY OF NAFLD IN MORBID OBESITY AND REDUCED BY BARIATRIC SURGERY

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Background and Aims: Macrophages play an important role in non-alcoholic fatty liver disease (NAFLD). Soluble CD163 (sCD163) is a specific marker of macrophage activation. We aimed to measure sCD163 in morbidly obese patients with varying degrees of NAFLD before and after bariatric surgery (BS).
Methods: Demographic, clinical and biochemical data, and plasma sCD163 measured by ELISA, of 196 patients were collected preoperatively and 3, 6 and 12 months after BS leading to significant weight loss. Peroperative liver biopsies were assessed for the NAFLD Activity Score (NAS), Kleiner fibrosis score and the FLIP algorithm. In a subset, CD163 immunohistochemistry and real-time quantitative PCR for CD163 mRNA were performed.
Results: sCD163 was higher in patients with NAS ≥5 compared to those with NAS <5 [2.4 (2.0–3.1) vs. 1.9 (1.5–2.3) mg/L, p < 0.001] and in patients with bridging fibrosis (F ≥3) compared to lower fibrosis stages [2.6 (2.0–4.9) vs. 2.0 (1.5–2.4) mg/L, p = 0.001]. Preoperative sCD163 was independently associated with both the NAS (p = 0.002) and the fibrosis score (p = 0.024). sCD163 decreased after BS and was greatly reduced after 12 months, more rapidly so in patients with NAS ≥5 (p < 0.001) and NASH according to the FLIP algorithm (p = 0.03). Immunohistochemistry showed CD163-positive macrophages aligning fat-laden hepatocytes and forming microgranulomas in patients with NASH. CD163 mRNA expression did not vary with NAS.
Conclusions: sCD163 increased in parallel with the severity of NAFLD in morbid obesity, indicating macrophage activation. Bariatric surgery reduced sCD163 even in patients with severe liver injury and fibrosis, suggesting full reversibility of macrophage activation associated with improved insulin sensitivity.

P1056
INSULIN RESISTANCE AND LIVER DAMAGE ARE ASSOCIATED WITH EARLY SIGNS OF LEFT VENTRICULAR SYSTOLIC DYSFUNCTION IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE, INDEPENDENTLY OF DIABETES, HYPERTENSION AND DYSLIPIDEMIA

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Background and Aims: Nonalcoholic Fatty Liver Disease (NAFLD) has been associated with subclinical cardiovascular disease (CVD).

This study was undertaken to evaluate the relationship between metabolic parameters, histologic features and parameters of cardiac morphology and function in NAFLD subjects.
Methods: Nineteen non-diabetic, non-dyslipidemic, non-hypertensive patients with biopsy-proven NAFLD (17 men, age 41±8 years, BMI 26.8±3 kg/m^2) and 9 healthy controls (5 men, age 30±2 years, BMI 22.5±2 kg/m^2) underwent transthoracic echocardiography and cardiac MRI to evaluate cardiac morphology and function. Endogenous glucose production (EGP) and lipolysis were assessed by stable isotope tracers. Hepatic Insulin resistance (IR) as EGP x fasting insulin, Oral Glucose Insulin Sensitivity (OGIS), adipo-IR as free fatty acids (FFAs) x fasting insulin were calculated.
Results: Despite the absence of diabetes, hypertension and overt dyslipidemia, NAFLD patients had significantly higher concentration of FFAs than controls ($p < 0.05$) and higher total saturated and monounsaturated levels ($p < 0.05$). In NAFLD basal Hepatic-IR (NAFLD vs controls: 92±34 vs 52±18 umol/min kg*mU/L) and Adipo-IR (NAFLD vs controls: 21±10 vs 11±5 mmol/L*mU/L) were significantly increased ($p < 0.03$ for all NAFLD vs controls) and OGIS significantly reduced (NAFLD vs controls: 11.0±1.64 vs 13.1±1.1 mg/kg min, $p = 0.005$). The end-systolic LV diameter (30.4±3.7 vs 27.2±3.5 mm, $p = 0.044$) was significantly higher in patients than in controls, suggesting subclinical systolic dysfunction. In NAFLD patients, both hepatic-IR and adipo-IR directly correlated with MRI end-systolic LV volume (ESV) ($r = 0.63$, $p = 0.004$ and $r = 0.54$, $p = 0.018$, respectively), while OGIS was inversely related to end-systolic LV diameter ($r = -0.48$, $p = 0.037$) and ESV ($r = -0.48$, $p = 0.036$). At liver biopsy, steatosis ≥33% was associated with increased ESV ($p = 0.047$), suggesting early systolic dysfunction. Similarly, ESV was increased in patients with fibrosis (69.2±16.9 vs 94.5±31.4 cc, $p = 0.018$), whereas the ejection fraction (51±7 vs 59±7%, $p = 0.034$) and cardiac index (2.9±0.7 vs 3.8±0.9, $p = 0.03$) were significantly reduced.
Conclusions: In NAFLD subjects metabolic derangements and histological features are associated with subclinical systolic LV dysfunction, independently of diabetes, hypertension and dyslipidemia.
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P1057
NON-ALCOHOLIC LIVER FATTY DISEASE AND SUBCUTANEOUS ADIPOCYTE SIZE IN HUMAN MORBID OBESITY

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Background and Aims: Non-alcoholic liver fatty disease (NAFLD) is one of common liver comorbidities in obesity and is associated with increased liver related morbidity and mortality. During adipose tissue expansion in obesity, adipocyte hypertrophy significantly influences adipocyte biology and is subsequently associated with obesity-associated comorbidities such as type 2 diabetes. However its link with liver injury in obesity remains unclear. The aim of this study was to investigate the relationships between liver histological injuries of NALFD and subcutaneous adipocyte size in morbid obesity.
Methods: 78 morbidly obese subjects (BMI 45.9±6.8 kg/m^2) candidate for bariatric surgery were recruited. The adipocyte diameters of subcutaneous adipose tissue (scAT) from needle biopsy, bioclinical parameters and liver stiffness measurement (LSM) measured by FibroScan before the surgery as well as per-operative liver biopsy (LB) samples were collected. LB was assessed by a single anatomo-pathologist. Fibrosis stage, steatosis and ballooning grades and the presence of NASH were assessed using the SAF score [Pierre Bedossa. *Hepatology* 60, 565–575 (2014)].
Results: LB evaluation showed that: 19 patients had NAFLD and 23 had NASH. Patients distribution was as follows: steatosis: (S0, n = 19; S1, n = 26; S2, n = 21; S3, n = 12); ballooning: (G0, n = 45; G1, n = 23; G2, n = 10); fibrosis: (F0, n = 46; F1, n = 21; F2, n = 6; F3, n = 4; F4, n = 1). NASH (123.58±14.1 μm) and NAFLD (116.92±9.06 μm) patients had larger adipocytes compared to those whose liver were normal (113.13±6.83 μm). Adipocyte diameter was associated with liver fibrosis (rho=0.35, $p = 0.003$), steatosis (rho=0.36, $p = 0.004$), ballooning (rho=0.439, $p < 0.001$) and LSM (rho=0.397, $p = 0.003$). Among them, ballooning grades was independently correlated with adipocyte diameters ($p < 0.001$). In addition, concerning BMI at 20 years old, NASH (26.5±6.7 kg/m^2) and NALFD (29.0±9.2 kg/m^2) patients were significantly less obese at early adulthood compared to those whose liver were normal (32.9±7.1 kg/m^2).
Conclusions: Our study shows that having a larger adipocyte size is associated with severe liver histological injuries, especially ballooning, a key feature of NASH in morbidly obese subjects. On contrary, adipose tissue expansion at early adulthood might be less deleterious to liver. Further studies are required to evaluate whether the decrease of adipocyte size after weight loss induced by bariatric surgery is associated with improved liver injury.

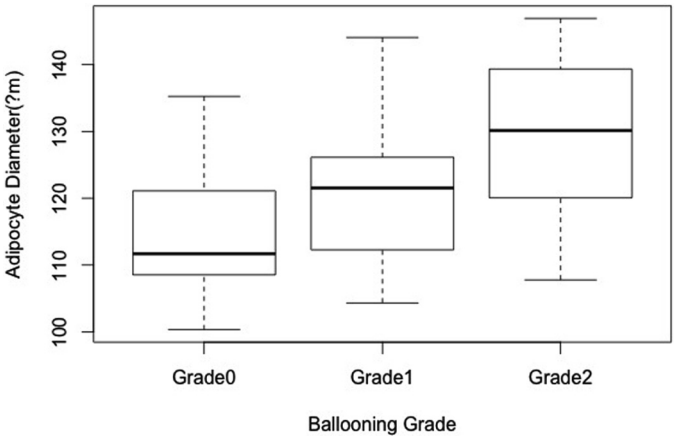

Figure: Boxplot of adipocyte diameter according to liver ballooning grade.

P1058
INCREASED EPICARDIAL FAT AND EARLY SIGNS OF IMPAIRED DIASTOLIC AND SYSTOLIC LEFT VENTRICULAR FUNCTION IN NON-DIABETIC, NON-DYSLIPIDEMIC, NORMOTENSIVE PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Nonalcoholic Fatty Liver Disease (NAFLD) has been associated with subclinical cardiovascular disease (CVD) and increased CVD morbidity and mortality. This study was undertaken to (1) ascertain whether NAFLD patients have abnormal epicardial fat, left ventricular (LV) morphology and function, (2) determine the relative contribution of epicardial, visceral and hepatic fat to LV dysfunction.
Methods: Nineteen non-diabetic, non-dyslipidemic, non-hypertensive patients with biopsy-proven NAFLD (17 men, age 41±8 years, BMI 26.8±3 kg/m^2) and 9 healthy controls (5 men,

age 30±2 years, BMI 22.5±2 kg/m^2) underwent transthoracic echocardiography and cardiac MRI to quantify epicardial fat and cardiac morphology and function. An abdomen MRI was performed in all subjects to quantify visceral and hepatic fat.
Results: The end-systolic LV diameter (30.4±3.7 vs 27.2±3.5 mm, p = 0.044) and the left atrial area (19.6±3.3 vs 14.9±2.5 cm^2, p = 0.02) were significantly higher in patients than in controls. Indices of LV diastolic function, namely the early diastolic septal and lateral mitral annular velocities, were significantly impaired in NAFLD (9.7±2.6 vs 12.9±2.57 and 13.3±3.5 vs 16.4±1.6 cm/s, vs controls respectively; p < 0.03 for both). Similarly, the ratio of early to late diastolic mitral inflow (E/A ratio) was significantly reduced in patient than in controls (1.2±0.2 vs 1.5±0.2, p = 0.004) suggesting an early diastolic dysfunction. Epicardial fat in NAFLD patients was significantly increased compared to controls, both at echocardiography and at MRI (5.93±2.25 vs 0.26±0.5 mm, 228.1±112.8 vs 66.8±25.2 cm^3, respectively, p = 0.001). In NAFLD patients, but not in controls, epicardial fat positively correlated with end-systolic LV diameter (r = 0.46, p = 0.046) and inversely with the ejection fraction (EF). Epicardial fat was tightly related to visceral fat (r = 0.58, p = 0.03), but not to hepatic fat. Visceral fat inversely correlated with EF, whereas hepatic fat directly correlated with the end systolic LV volume (r = 0.63, p = 0.004).
Conclusions: Non diabetic, non-dyslipidemic, normotensive NAFLD patients show early signs of both diastolic and systolic LV dysfunction. Epicardial, visceral and hepatic fat are increased and associated with parameters of early systolic dysfunction.
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P1059
SEVERITY OF CORONARY ARTERY DISEASE IS ASSOCIATED WITH NON-ALCOHOLIC FATTY LIVER DISEASE
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Background and Aims: Recent studies showed a significant association between non-alcoholic fatty liver disease (NAFLD) and coronary artery disease (CAD). Previous studies have reported good diagnostic accuracy of transient elastography (TE) for the non-invasive assessment of liver fibrosis, and of controlled attenuation parameter (CAP) for assessment of hepatic steatosis. The aim of the present study was to evaluate whether presence and severity of CAD are associated with presence and severity of NAFLD.
Methods: Patients who underwent invasive coronary angiography were enrolled in this prospective study between December 2011 and October 2014. All patients received at least 10 TE and CAP measurements using the FibroScan® M-probe (Echosens, Paris, France). Quality criteria for TE (10 successful measurements, IQR/median ≤0.30) and CAP (10 successful measurements) were respected. Clinical relevant CAD (rCAD) was defined as presence of stenosis with at least 75% reduction of the luminal diameter with the need for coronary revascularization. NAFLD was determined by CAP ≥234 dB/m, corresponding to >5% hepatocytic fat content. Non-alcoholic steatohepatitis (NASH) was determined by TE-values ≥7.9 kPa in the presence of hepatic steatosis and absence of echocardiographic signs of congestive or right-sided heart failure. Significance level was specified as p < 0.05.
Results: 576 patients were enrolled in the study. 71 (12.3%) patients were excluded due to CAP-failure, 125 (21.7%) due to TE-failure. Therefore, a total of 505 patients [78% male; mean age 66±12 years; mean body mass index 27.3±4.6 kg/m^2] were available for analysis of NAFLD, 451 for analysis of NASH. 356/505 (70.5%) patients had rCAD, 361/505 (71.5%) were diagnosed with NAFLD, and 35/451 (7.8%) with NASH. Patients with rCAD had significantly higher mean CAP-values than patients without rCAD (273±61 vs. 261±66 dB/m; p = 0.038) and tended to higher degrees of steatosis (p = 0.037). While NAFLD was significantly more often diagnosed in patients with rCAD as compared to patients without (75.0% vs. 63.1%, p = 0.0094), no significant difference was found for the diagnosis of NASH (7.9% vs. 7.5%, p = 1.0).
Conclusions: Our findings demonstrate that clinical relevant CAD is frequently associated with the presence of NAFLD. Besides cardiological follow-up, these patients should also receive hepatological monitoring of NAFLD to enable the diagnosis of NASH and prevent its potential sequelae.

P1060
MINIMALLY INVASIVE DIAGNOSIS OF NAFLD AND DISCRIMINATION BETWEEN NAFL AND NASH BY AUTOANTIBODY SIGNATURES
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Background and Aims: Reliable diagnosis and discrimination of non-alcoholic fatty liver (NAFL) and steatohepatitis (NASH) still requires liver biopsy. Moreover, auto-antibodies add to differential diagnostic challenges in NAFLD/NASH. In this study, we aimed to identify novel serum biomarkers diagnostic for NAFLD and distinctive of NAFL/NASH.
Methods: A pilot cohort of patients with biopsy proven NAFLD was compared to age- and sex-matched healthy controls. In a non-hypothesis driven approach, serum samples were analysed for the presence of novel auto-antigens using a bead-based Luminex technology.
Results: The cohort consisted of 48 NAFLD patients (NAFL n = 34: 75% male; median±SD age: 42±12.4 y; NASH n = 14: 50% male; median±SD age: 52±13 y; p = n.s.) and 20 healthy controls (60% male; median±SD age: 48±15 y). Univariate analysis identified several antigens distinctive of NAFLD (best AUC: 0.865 [CI: 0.744; 0.987], sensitivity: 85.2% [CI: 0.699; 1.000], specificity: 60.0% [CI: 0.350; 0.850]), as well as discriminating between NAFL and NASH (best AUC: 0.821 [CI: 0.683; 0.959], sensitivity: 75% [CI:0.656; 0.859], specificity: 60% [CI: 0.219; 0.981]). According to multivariate analysis, 3 novel single antigens predictive of NAFLD were identified, so far known to be involved in cell growth, cell differentiation, and hepatic lipid metabolism, with AUC, sensitivities and specificities between 0.759–0.791, 76–84%, and 54–65%, respectively.
Conclusions: Using a bead-based Luminex screen we identified single serum markers discriminative for NAFL vs. NASH. Additionally, as confirmed by multivariate analysis, 3 novel immunological biomarkers diagnostic for NAFLD were identified. These markers may provide novel mechanistic and prognostic insights into NAFLD and may be more stable than metabolomic signatures.

P1061

THE MACROPHAGE ACTIVATION MARKER SCD163 AND THE APOPTOSIS MARKER CYTOKERATIN-18 ARE BOTH PREDICTORS OF DISEASE SEVERITY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Macrophages play an important role in non-alcoholic fatty liver disease (NAFLD). Cytokeratin-18 (CK-18) is an extensively investigated apoptosis biomarker of NAFLD and NASH. We hypothesized that a specific macrophage activation marker soluble (s)CD163, and CK-18 would both contribute to the prediction of disease activity and fibrosis in NAFLD.
Methods: Associations of sCD163 and CK-18 with biochemical and histological measures of hepatic inflammation and fibrosis were investigated in two independent cohorts of 171 and 174 NAFLD patients. Demographic, clinical, biochemical, and metabolic data were recorded at the time of liver biopsy; sCD163 and CK-18 were measured by specific ELISAs.
Results: Both sCD163 and CK-18 increased in parallel with the severity of NAFLD and were independently associated with the Kleiner fibrosis score and the steatosis score in both cohorts. In multiple logistic regression analysis, both sCD163 and CK-18 were significant predictors of severe activity (NAS ≥5) and advanced fibrosis (F ≥3). A fibrosis score based on sCD163 and CK-18 improved the prediction of advanced fibrosis than either sCD163 or CK-18 alone and showed excellent Areas Under the Receiver Operating Characteristics curve (AUROCs) in both the estimation [0.88 (95% CI: 0.82–0.94)] and the validation [0.83 (95% CI: 0.75–0.92)] cohorts. In contrast, the prediction of NAS ≥5 was not improved by a score based on both variables compared to the one based on sCD163 alone.
Conclusions: Both sCD163 reflecting macrophage activation and the apoptosis marker CK-18 are independently associated with liver injury and fibrosis in NAFLD. Combining sCD163 and CK-18 as markers of different pathophysiological events in a predictive model improves the prediction of advanced fibrosis.

P1062

IRON METABOLISM IN CHILDREN WITH NONALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Nonalcoholic fatty liver disease (NAFLD) nowadays is regarded as the most frequent cause of liver pathology. The studies on adults population have suggested that iron overload plays a significant role in pathogenesis of NAFLD and progression from simple steatosis to nonalcoholic steatohepatitis (NASH). Therefore, the aim of the study was to evaluate serum concentration of selected parameters of iron metabolism in obese children with NAFLD.
Methods: The study comprised 111 obese children (age range 7–17) with the initially suspected liver disease (hepatomegaly and/or increased ALT activity and/or liver steatosis in ultrasound). Viral hepatitis (HCV, HBV), autoimmune (AIH) and selected metabolic liver diseases (Wilson's disease, alfa-1-antitrypsin deficiency, cystic fibrosis) were excluded. The degree of liver steatosis in ultrasound (USG) was graded according to Saverymuttu scale. The total intrahepatic lipid content was assessed by magnetic resonance proton spectroscopy (^{1}HMRS). Serum iron, ferritin, hepcidin levels, total iron-binding capacity (TIBC), soluble transferrin receptor (sTFR) was measured in all participants. In 45 patients hemojuvelin (HJV) level was also assessed. In addition, AST to Platelet Ratio Index (APRI) was calculated and used as a noninvasive test for predicting liver fibrosis.
Results: NAFLD was confirmed in 39 children. Serum ferritin, hepcidin levels and TIBC were significantly higher in children with NAFLD than in obese patients without NAFLD. NAFLD children demonstrate also significantly higher ALT and GGT activities, APRI, insulin resistance (HOMA-IR), BMI, waist circumferences and total amount of lipids in ^{1}HMRS. We found significant correlations of serum iron ($r = 0.19$), ferritin ($r = 0.31$), hepcidin ($r = 0.37$) levels as well as TIBC ($r = 0.25$) with liver injury (ALT/GGT activity). Correlation was also demonstrated between APRI and ferritin ($r = 0.41$), hepcidin (0.31) and TIBC (0.24). Moreover, TIBC correlated with the degree of liver steatosis in USG ($r = 0.23$).
Conclusions: Elevated serum concentrations of selected parameters of iron metabolism observed in children with NAFLD and their association with liver injury and APRI suggest the role of iron metabolism in pathogenesis of fatty liver and progression to NASH.

P1063

EVALUATION OF TRANSIENT ELASTOGRAPHY (FIBROSCAN®) FOR THE MEASUREMENT OF LIVER STIFFNESS IN MORBIDLY OBESE PATIENTS BEFORE BARIATRIC SURGERY

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Background and Aims: *Background:* Non-alcoholic fatty liver disease (NAFLD) has a rising prevalence in the western world. In severely obese patients undergoing bariatric surgery the estimated prevalence is about 90%, and a substantial number of these patients may have end-stage liver disease with liver cirrhosis. Fibroscan as a non-invasive diagnostic tool to measure liver stiffness has a good accuracy in various liver diseases. In severely obese patients few data exist about technical feasibility and accuracy of liver stiffness measurement by Fibroscan®. *Aims:* To analyse non-invasive liver stiffness measurement by Fibroscan® in morbidly obese patients undergoing bariatric surgery compared to liver histology.
Methods: Prospective patient cohort with 158 morbid obese NAFLD patients. Fibroscan measurement was performed with XL probe. Fibroscan® examination was successful with 10 valid measurements, success rate >60% and interquartile range of measurements (IQR)/median (M) of liver stiffness ≤0.3 or IQR/M ≥0.3 with a median of liver stiffness <7.1 kPa.
Results: In the cohort 158 NAFLD patients were included and 79 patients had liver biopsies (NAFL n = 55, NASH n = 24). The patients were morbidly obese with a mean BMI of 49.1 kg/m^2 (SD 10.4). In 43.4% Fibroscan® measurement with XL-probe was technically feasible. Failure of Fibroscan® measurement was more frequent in patients with a higher BMI, a higher weight and a higher NAFL fibrosis score ($p < 0.05$). In patients with valid Fibroscan® measurement, 10 patients with NASH had a significantly higher

liver stiffness 9.3±5.5 kPa in comparison to 25 patients with NAFL 6.3±2.6 kPa ($p < 0.05$). Patients with early fibrosis (F1 and F2) could not be differentiated by Fibroscan® measurement from patients with no fibrosis.
Conclusions: Non-invasive liver stiffness measurement is feasible in severe obese patients with XL probe. NASH patients could be differentiated from patients with NAFL. Transient elastography is useful in clinical evaluation before bariatric surgery.

P1064
ASSOCIATION BETWEEN PNPLA3 I148M POLYMORPHISM AND NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN THE INDIAN CONTINENT
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Background and Aims: Single nucleotide polymorphism characterized by a C-to-G change encoding an isoleucine-to-methionine substitution at 148 in the human, patatin-like phospholipase 3 (PNPLA3) has been reported to be associated with non-alcoholic fatty liver disease (NAFLD) and advanced liver disease. We investigated the genotype frequency of PNPLA3 rs738409 in NAFLD patients and its association with different biochemical and histological features.
Methods: A hospital-based cross sectional study was conducted on NAFLD patient attending OPD at tertiary care centre in New Delhi to determine the distribution of PNPLA3 genotypes among NAFLD. Liver biopsy was done in 216 patients. We analysed the rs738409 polymorphism by TaqMan assay in all NAFLD patients and assessed its association with biochemical and histological features of NASH.
Results: A total of 310 NAFLD patients were included. Simple fatty liver was seen in 124 patients (40%) (mean age 40.9±14 years, male 73.4 5%, mean BMI 25.8±5.7 kg/m^2, lean 33.3%). There were 120 patients (38.7%) in precirrhotic NASH (Mean age 38.2±11.8 years, male 85.8%, mean BMI 24.8±5 kg/m^2, lean 42%) and 66 patients (21.3%) in NAFLD related cirrhosis (mean age 52.6±11.3 years, male 83.3%, mean BMI 25.6±4.68 kg/m^2, lean 35.2%). PNPLA3 genotypes were CC type in 121 (39%), CG type in 88 (28.40%) and GG type in 101 (32.60%) in all NAFLD patients. Prevalence of homozygous (GG) type was 33/124 (26.6%) in fatty liver, 31/120 (25.8%) in NASH and 37/66 (56.1%) in cirrhotic patients (p value <0.001). There was no influence of PNPLA 3 genotypes on clinical (BMI, AST, ALT, insulin, triglycerides, VLDL, cholesterol, liver stiffness measurement) as well as histological features (steatosis, ballooning, lobular inflammation and fibrosis) in precirrhotic NASH (p value >0.5). There was also no association between the presences of PNPLA 3 in lean (BMI <23 kg/m^2) versus obese (>23 kg/m^2) NAFLD patients.
Conclusions: GG type was significantly more common in NAFLD related cirrhotic patients as compared to simple fatty liver and NASH. The PNPLA3 rs738409 polymorphism neither correlated with histological features like steatosis severity, hepatocellular ballooning, lobular inflammation and fibrosis nor with biochemical parameters of NASH patients.

P1065
LOVASTATIN'S AND PENTOXIFYLLINE'S EFFECTS IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS
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Background and Aims: Today, there is no ideal treatment for nonalcoholic steatohepatitis (NASH). We performed a multicentric prospective study in order to assess the lovastatin and pentoxifylline efficiency administrated in patients with NASH
Methods: A number of 87 patients with NASH were included in the study, out of which 59 were with NASH and dislypidemia and were treated with lovastatin (10 mg/day), and 28 patients were with NASH, but without dislypidemia and were treated with with pentoxifylline, 400 mgx3/day.
Results: Regarding the lovastatin-treated group, the following results were obtained: the level of aspartate aminotransferase (AST) decreased after the first and second month of treatment ($p = 0.0196$, $p = 0.032$); the level of alanine aminotransferase (ALT) decreased after the first and second month of treatment ($p = 0.0335$, $p = 0.021$). The gamma-glutamyl transferasis (GGT) level decreased during the two months of treatment ($p = 0.079$, respectively $p = 0.253$). The level of cholesterol decreased after the first and second month of treatment ($p = 0.00029$, respectively $p = 0.00028$). The APRI score of liver fibrosis in the 3 months of treatment, decreased from the average initial value of 0.188 to 0.142, respectively to 0.102 after the second month of treatment ($p = 0.030$).
Regarding the pentoxifylline-treated group, the following results were obtained: the level of AST decreased after the first month and second month of treatment ($p = 0.018$, $p = 0.16$); the level of ALT significantly decreased after the first and second month of treatment ($p = 0.033$, $p = 0.126$). The GGT level decreased after the first and the second month of treatment ($p = 0.107$, respectively $p = 0.123$). The APRI score decreased from the average initial value of 0.1587 to 0.1135 after the first month of treatment and to 0.133 after the second month of treatment ($p = 0.022$).
Conclusions: Both drugs significantly decreased the levels of transaminases in patients with NASH. Lovastatin reduced the cholesterolemia in the dislypidemic patients. The decrease of the APRI score suggests that both drugs may have benefic effects on the liver histology, too. Our research pleads for the individual treatment in patients with NASH, taking into account the present components of the metabolic syndrome.

P1066
NAFLD, FETUIN-A AND ATHEROSCLEROSIS BURDEN: DOES VASCULAR TOPOGRAPHY MATTER?
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Background and Aims: The pathogenic role of Fetuin-A (F-A) in atherosclerosis (Athero) burden in NAFLD is poorly defined. We assessed F-A serum levels and NAFLD prevalence in patients (pts) affected by Athero in different anatomical regions.
Methods: 149 consecutive pts with established Athero were recruited: 45 with coronarography-detected >50% coronary stenosis, 23 with severe carotid stenosis, 55 with symptomatic Athero of lower limbs and 26 with thoracic or abdominal aortic aneurysm. NAFLD diagnosis based on ultrasonography and exclusion of competing etiologies. Fetuin-A measured with ELISA. Multivariate analyses were conducted to assess the independent correlates of coronary artery disease (yes/no), NAFLD (yes/no) and F-A (continuous values).
Results: 78% of cases were male, mean age was 71±10 yrs. Mean BMI was 27±4 kg/m^2, 61% of pts had diabetes, 93% were hypertensive, 62% used lipid-lowering drugs and 50% had metabolic syndrome. NAFLD was detected in 54% of pts; mean F-A levels were 260±115 μg/ml. Coronary pts were significantly younger ($p = 0.008$), had lower HOMA ($p = 0.019$), less frequently diabetes ($p = 0.045$) and NAFLD (42% vs 60% $p = 0.050$), but higher diastolic

pressure ($p < 0.001$), total cholesterol ($p = 0.023$) and F-A levels (374 ± 124 vs 243 ± 63 $p < 0.001$) than non-coronary pts. NAFLD pts were significantly younger ($p = 0.044$), had higher BMI ($p < 0.001$), total cholesterol ($p = 0.001$) and triglycerides ($p = 0.002$) and more frequently metabolic syndrome ($p = 0.004$) than non-NAFLD pts. F-A was positively correlated with coronary artery disease, BMI, total cholesterol, diastolic and systolic pressure, metabolic syndrome and albumin and negatively with age and platelet count. At multivariate analysis: (1) coronary pts had higher F-A levels and less frequently NAFLD than non-coronary pts (independent of demographic and metabolic covariates); (2) NAFLD was positively associated with F-A levels, after adjustment for vascular topography; (3) F-A levels were independently positively associated with coronary artery disease and NAFLD and negatively with ALT and platelet count (independent of demographic and metabolic covariates).
Conclusions: The pathogenic interplay of NAFLD, F-A and Athero burden likely varies based on vascular topography.

P1067

ASSOCIATIONS BETWEEN ANTIDIABETIC AND STATIN THERAPY AND LIVER HISTOLOGY IN PATIENTS WITH TYPE 2 DIABETES AND NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Type 2 diabetes mellitus (DM) is a risk factor for progressive non-alcoholic fatty liver disease (NAFLD). Drugs commonly prescribed in DM patients (pts) affect insulin sensitivity and lipid profile and may impact liver histology. We studied if antidiabetic agents or statins were associated with non-alcoholic steatohepatitis (NASH) and significant fibrosis (SF).
Methods: A cross-sectional study of 346 diabetic pts with biopsy-proven NAFLD, 138 referred to a tertiary liver center and 208 morbidly-obese pts candidate to bariatric surgery. DM was defined as fasting glucose ≥7 mmol/l or HbA1c ≥6.5% and/or the use of antidiabetic treatment. NASH was defined according to the FLIP algorithm and SF as F2–4 Kleiner's stages.
Results: There were 60% women, mean age was 52 ± 11 yrs, median BMI 42 [21–74] kg/m^2, 76% had arterial hypertension (HTN); 54% hypertriglyceridemia, 80% low HDL-cholesterol, mean HbA1c was 7.4 ± 1.3%. 84% of pts were on antidiabetic therapy (sulfonylureas 33%, metformin 74% and insulin 24%) and 45% on statins. NASH and SF were present in 57% and 48% of pts, respectively. Statin-treated pts were older, more frequently male, hypertensive (90% vs. 64%, $p < 0.001$), dyslipidemic, with a poorer glycemic control (HbA1c 7.7 ± 1.5% vs. 7.1 ± 1.1%, $p > 0.001$) despite more frequent antidiabetic therapy (sulfonylureas 42% vs. 25%, $p = 0.001$; metformin 85% vs. 65%, $p < 0.001$; insulin 33% vs. 17%, $p = 0.001$) than those without statins; however, the prevalence of NASH (57% vs. 56%, $p = 0.868$) and SF (48% vs. 48%, $p = 0.945$) was not different between statin users and nonusers. NASH was more common in pts on metformin or insulin than in those not treated with these drugs (60% vs. 47%, $p < 0.03$; 68% vs. 53%, $p < 0.02$, respectively). SF was more common in those treated with sulfonylureas (57% vs. 44%, $p = 0.03$). Multivariate analyses including age, sex, BMI, HTN, dyslipidemia, glycemic control, antidiabetic and statins medications, ALT, GGT and cohort origin confirmed independent associations between insulin use and NASH [OR (95% CI): 2.3 (1.1–4.6), $p = 0.020$] and sulfonylureas use and SF [2.0 (1.1–3.6), $p = 0.028$]. Moreover, use of statins was independently and negatively associated with both NASH [0.6 (0.3–1.0), $p = 0.057$] and SF [0.5 (0.3–0.9), $p = 0.033$].
Conclusions: In diabetic pts with NAFLD, insulin and sulfonylureas appear to be associated with NASH and SF while statin use appears protective. These associations are independent of the baseline severity of the clinical condition. A wider use of statins may be warranted in these pts.

P1068

METFORMIN TREATMENT AMELIORATES LIVER INJURY INDICATING FATTY LIVER IN POLYCYSTIC OVARY SYNDROME

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Background and Aims: Polycystic ovary syndrome (PCOS) is associated with obesity and insulin resistance (IR), two key features of the metabolic syndrome. Non-alcoholic fatty liver disease (NAFLD) represents the liver manifestation of the metabolic syndrome. Its progressive form, non-alcoholic steatohepatitis (NASH), comprises a major health risk for affected patients. Metformin (MF) as insulin sensitizing agent leads to a decrease of hepatic IR and could thus possibly ameliorate NASH. The caspase 3-cleaved fragment of cytokeratin 18 (CK18), termed M30, has been established as a serum marker for NASH. This study was conducted to evaluate the influence of MF on serologic NASH in PCOS patients.
Methods: In 89 PCOS patients (age: 28.2 ± 5.2 yrs, BMI: 32.1 ± 8.8 kg/m^2), metabolic parameters, liver enzymes, and M30 were assessed at baseline and after six months MF treatment. Patients with IR at baseline were subdivided in one group with dissolved IR (PCOS-exIR) and persistent IR (PCOS-PIR) after treatment and compared to an initially insulin sensitive PCOS-group (PCOS-C).
Results: Reduction of liver enzymes by MF treatment was observed independently from achieved body weight loss in the PCOS-C and PCOS-exIR in comparison to the PCOS-PIR group. This was associated with decreasing prevalence of liver injury indicating fatty liver (−19.4 resp. −12.0% vs. 7.2%, $Chi^2 = 29.5$, $p < 0.001$). No change in mean M30 levels and NASH-prevalence was observed. In PCOS-PIR, ALT levels increased significantly accompanied by a nominal, not significant M30 increase.
Conclusions: MF improves liver enzymes in subgroups of PCOS patients without lowering hepatic apoptosis markers. Though, individuals with no improvement of IR exhibited a non-significant increase of liver enzymes and M30. This might suggest a stabilizing effect of MF on NAFLD, inhibiting progression of the disease. Further studies with more participants and a longer follow up period (>1year) are needed to elucidate the impact of MF on NAFLD in PCOS patients.

P1069

THE ASSOCIATION BETWEEN FATTY LIVER STATUS CHANGE AND INCIDENCE OF DIABETES MELLITUS IN JAPAN

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Background and Aims: An association between fatty liver (FL) and diabetes mellitus (DM) has been reported. However, it is still uncertain if a change in fatty liver status affects DM incidence. The aim of our study was to assess the risk of future DM incidence in people whom fatty liver status had changed.
Methods: Eligible participants were those who had a health check in 2000, 2001, and at least once between 2002 and 2013. Those who had positive HBs antigen, positive HCV antibody, or DM in 2000 or 2001 were excluded, leading to the eligible 12,904 participants. The participants were divided into 4 groups; (1) "Improved FL" group (n = 234), which had FL in 2000 and no FL in 2001; (2) "Sustained FL" group (n = 1,146), which had FL in both 2000 and 2001; (3) "Newly developed FL" group (n = 760), which had no FL in 2000 but had FL in 2001; (4) "Non-FL group" (n = 10,764), which had no FL both in 2000 and 2001. Fatty liver was diagnosed using ultrasonography. DM was ascertained by fasting plasma glucose ≥126 mg/dL, HbA1c ≥6.5%, self-reported physician-diagnosed DM, or having any medication for DM. The outcome was cumulative incidence of DM during 2002–2013. Logistic regression model was used to estimate crude and multivariate odds ratios (ORs) and confidence interval (CI) for the association between DM incidence and the groups. Age, sex, family history of DM, overweight, hypertension, dyslipidaemia, frequency of exercise and smoking status in 2000 were adjusted in the multivariate model.
Results: During the follow-up period, cumulative incidence of DM was as follows: 2/234 (0.85%) in "improved FL", 35/1,146 (3.05%) in "sustained FL", 22/760 (2.89%) in "newly developed FL", and 50/10,764 (0.46%) in "non-FL". The multivariate ORs were 1.507 (95% CI 0.368–6.351) in "improved FL", 4.830 (95% CI 2.717–8.588) in "newly developed FL", and 5.024 (95% CI 2.977–8.479) in "sustained FL", compared with "non-FL". Comparing with "improved FL", multivariate ORs were 2.571 (95% CI 0.569–11.629) in "newly developed FL", and 3.492 (95% CI 0.825–14.787) in "sustained FL".
Conclusions: FL was a risk factor of future DM. The association between FL improvement and reduced incidence of DM appeared clinically significant, but was not statistically significant.

P1070

THE MARKER OF MACROPHAGE ACTIVATION SOLUBLE CD163 IS ASSOCIATED WITH LIVER HISTOLOGY IN CHILDREN WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Macrophages are involved in inflammation and fibrosis in non-alcoholic fatty liver disease (NAFLD). Soluble (s)CD163 is a specific marker for macrophage activation and in a previous study, sCD163 was increased in obese children with indices of NAFLD. We aimed to measure sCD163 in children with biopsy-proven NAFLD and associate it with clinical, biochemical and histological parameters of NAFLD.
Methods: We investigated 155 children with biopsy-proven NAFLD. Demographic, clinical and biochemical data were recorded. Liver biopsies were scored according to the NAFLD Clinical Research Network criteria. sCD163 was measured by ELISA.
Results: Soluble CD163 correlated with the body mass index standard deviation scores (r = 0.21, p = 0.05). sCD163 was associated with hepatocyte ballooning (rho=0.17, p = 0.03), but not steatosis or lobular inflammation. It showed a trend to association with the fibrosis score (rho=0.14, p = 0.09), and children with bridging fibrosis (F=3, n = 14) tended to have higher sCD163 compared to those with lower fibrosis stages [2.4 (IQR 1.7–2.9) vs. 1.8 (IQR 1.5–2.4) mg/L, p = 0.07]. The number of CD163 positive macrophages was associated with scores of steatosis, lobular inflammation and hepatocyte ballooning; however, the cell counts showed no association with sCD163 levels (r = 0.12, p = 0.24).
Conclusions: sCD163 reflecting macrophage activation was associated with histological parameters of liver injury and fibrosis, suggesting a role for macrophage activation in pediatric NAFLD. However, there was no association with CD163 immunohistochemistry.

P1071

THE ASSOCIATION BETWEEN SERUM LEVELS OF URIC ACID AND ALANINE AMINOTRANSFERASE IN A POPULATION-BASED COHORT

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Background and Aims: Elevated serum uric acid is frequently observed in patients with the metabolic syndrome. A strong correlation exists between this syndrome and nonalcoholic fatty liver disease (NAFLD). Therefore, we aimed to test the association between uric acid and elevated alanine aminotransferase (ALT) in a large population-based cohort.
Methods: A cross-sectional study using real-world data from a large public health organization in Israel (Maccabi Healthcare System). The population consisted of individuals aged 20–60 years old who underwent blood tests for ALT and uric acid for any reason during 1997–2012. Individuals with secondary liver disease, celiac and inflammatory bowl-disease were excluded. Subgroup analysis was performed in subjects who were diagnosed with fatty liver in the medical records (n = 2,628). This database includes medical history, diagnoses, patient consultations, prescription drug purchase, laboratory and imaging tests.
Results: The study population included 82,608 people (32.5% men, mean age 43.91±10.15 years). Subjects in the upper quartiles (>5.6 mg/dL) of uric acid level were significantly (P < 0.001) more likely to have a poorer metabolic profile in terms of glucose, serum lipids, diabetes and hypertension. Categorizing serum uric acid into deciles demonstrated a significant positive dose-response association with the rate of elevated serum ALT (P for trend <0.001). In multivariate logistic regression analysis, controlling for potential confounders, the association between uric acid and elevated ALT persisted both in the total population (OR = 1.18, 95% CI 1.10–1.28; OR = 1.49, 1.38–1.62; OR = 2.10, 1.93–2.29, for the 2nd, 3rd and 4th quartiles respectively compared to the 1st) and in the subgroup of subjects who were diagnosed with fatty liver (OR = 1.77, 1.22–2.57, for the 4th quartile compared to the 1st). With stratification by gender or BMI categories, the association between uric acid and elevated ALT was maintained in all categories.

Conclusions: Serum uric acid is independently associated with elevated ALT, as a surrogate for NAFLD, and thus may serve as a serum marker and should be further investigated as a risk factor for NAFLD.

P1072

THE INTERACTION BETWEEN VISCERAL ADIPOSE AND HEPATIC TISSUE AFFECTS LIVER INJURY IN NAFL AND NASH PATIENTS

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) and the more severe form non-alcoholic steatohepatitis (NASH) are the most common liver diseases in western countries. The interaction between liver and adipose tissue is fundamental for the development of NAFLD and NASH. Aim of this study was to investigate the interaction between adipose tissue and liver at the time of bariatric surgery.
Methods: Blood, visceral adipose tissue, and liver tissue samples were obtained from 40 (median age 46±8.6 y; 29 w/11 m; BMI 51.3±8.1 kg/m^2) morbidly obese patients undergoing bariatric surgery. Histopathological assessment of the biopsies was done according to the NAFLD activity score (NAS). Blood samples taken before surgery were analyzed for parameters of liver injury (M30, M65). Hepatic and adipose tissue mRNA levels of one gene for triacylglycerol regulation (CGI-58) and a central regulating gene for fatty acid storage and glucose metabolism (PPARγ2) were assessed by qrtPCR.
Results: CGI-58 mRNA expression was increased in adipose tissue of morbidly obese patients and showed a strong correlation to low density lipoprotein (LDL) in sera and with the NAS. In morbidly obese individuals mRNA of PPARγ2 was significantly upregulated in hepatic and adipose tissue. Moreover, PPARγ2 mRNA levels in adipose tissue were correlated significantly with hepatic PPARγ2 mRNA levels. The ratio of markers for apoptosis and necrosis (M30, M65) in serum also correlated significantly with mRNA levels of metabolic regulators in adipose tissue.
Conclusions: The presented data shows that adipose CGI-58 and PPARγ2 mRNA expression is associated on the one hand with mRNA expression in the liver and on the other hand with the grade of liver injury.

P1073

INTERLEUKIN 15 IN NONALCOHOLIC FATTY LIVER DISEASE AND OBESITY

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Background and Aims: *Background:* Non-alcoholic fatty liver disease (NAFLD) and obesity represent widespread pathologies associated with low-grade inflammatory processes. Experimental data suggest crucial role of anabolic cytokine interleukin 15 (IL-15) in NAFLD development. Y. Cepero-Donates et al. showed that increased secretion of IL-15 promotes fat accumulation in the liver stimulating hepatic inflammatory response in mice.
The aim was to investigate IL-15 concentration in patients with NAFLD associated with obesity depending on steatosis degree and anthropological parameters. The study included 32 patients with NAFLD associated with obesity, 31 normal weight patients with NAFLD and 26 normal weight volunteers without hepatic steatosis.
Methods: NAFLD diagnosis and assessment were performed by abdominal ultrasound examination. Steatosis degree was evaluated using hepatorenal index (HRI). For all NAFLD patients other causes of hepatic steatosis were excluded. Obesity was measured using body mass index (BMI) and waist circumference (WC). Concentration of IL-15 were measured using enzyme-linked immunosorbent assay kit.
Results: The results showed that IL-15 concentration was significantly increased in patients with NAFLD comparing to control group ($p < 0.05$). Concentrations of IL-15 observed in NAFLD patients with concomitant obesity were significantly higher than those measured in NAFLD patients with normal weight ($p < 0.05$). In all NAFLD patients IL-15 correlated with HRI supporting its role in hepatic fat accumulation. Furthermore, in patients with NAFLD there was significant correlations of IL-15 concentration with BMI ($p < 0.05$) and WC ($p < 0.05$).
Conclusions: Patients with NAFLD and concomitant obesity might have more significant proinflamatory status that could be caused by adipose tissue dysfunction and cytokine synthesis abnormalities. Increased synthesis of IL-15 observed in NAFLD obese patients supports its role in hepatic lipid accumulation found in experimental studies. Further investigations are needed to explore correlations of IL-15 with histological findings in NAFLD obese patients.

P1074

NO EVIDENCE FOR PLATELET HYPERACTIVITY IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: The mechanism of increased prevalence of cardiovascular disease in patients with non-alcoholic fatty liver disease (NAFLD) is unknown. Platelet activation has been implicated as a contributor of the increased risk of cardiovascular disease in the metabolic syndrome, but its role in NAFLD is unclear. We, therefore, assessed the platelet activation status in lean and obese individuals in comparison to patients with various histological severities of NAFLD.
Methods: Blood was drawn from 68 patients with biopsy-proven NAFLD (simple steatosis n = 24, NASH n = 22, and NASH cirrhosis n = 22), 20 lean controls (BMI <25 kg/m^2), 20 overweight controls (BMI >25 kg/m^2), and 15 patients with alcoholic (ASH) cirrhosis. Subjects with congenital coagulation disorders, recent infection (<2 weeks), anticoagulant or anti-platelet therapy, and recent transfusion with blood products were excluded. We studied basal and agonist-induced platelet activation using flow cytometry. In addition, we studied plasma levels of von Willebrand factor (VWF), the VWF-cleaving protease ADAMTS13, and the platelet activation marker soluble P-selectin.
Results: Basal platelet activation was comparable between lean and overweight controls, patients with NASH, and patients with NASH or ASH-related cirrhosis. There was only a slight increase in basal platelet activation in patients with simple steatosis compared to overweight controls. Agonist-induced platelet activation was decreased in patients with cirrhosis, most notably in patients with ASH-related cirrhosis, but similar between patients with non-cirrhotic NAFLD and controls. Plasma levels of VWF were increased in patients with NASH or ASH cirrhosis compared to all other groups; however levels were comparable between lean and overweight controls, patients with simple steatosis, and patients with NASH. ADAMTS13 levels were comparable between all patients and controls. Soluble P-selectin levels were mildly elevated in plasma from patients with NASH, NASH cirrhosis, or ASH cirrhosis compared to lean and overweight controls.

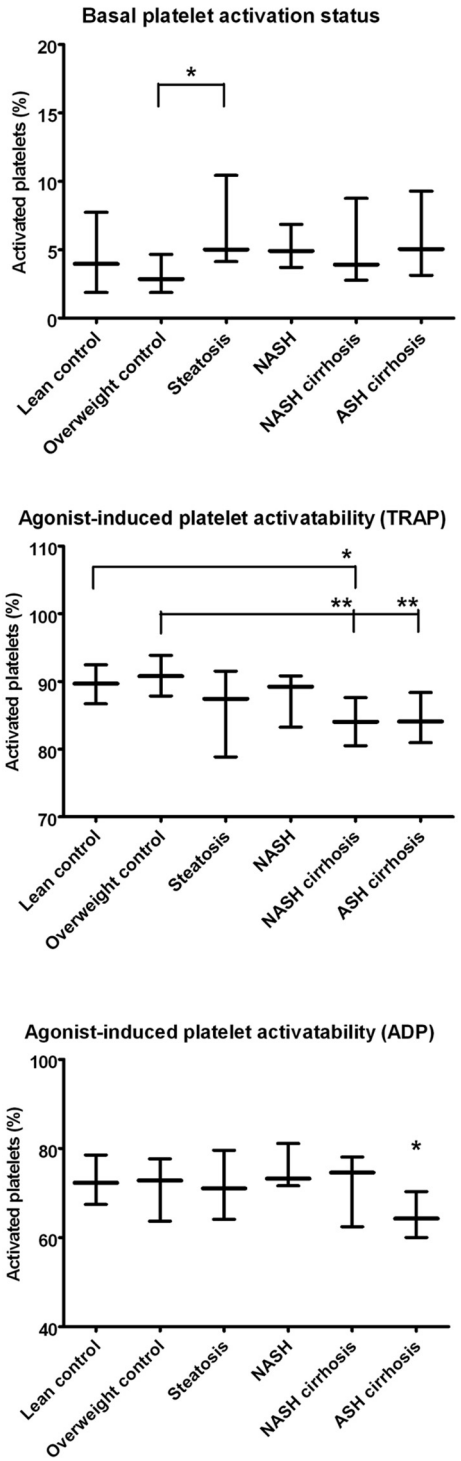

Figure 1. Basal and agonist-induced platelet activation in patients with various histologic severities of NAFLD and controls. The basal platelet activation status (A) and the agonist-induced platelet activatability in patients and controls using adenosine diphosphate (ADP) (B), or thrombin receptor activating peptide (TRAP) (C), as assessed by flow cytometry for P-selectin in patients with various histological severities of NAFLD and lean or overweight controls. Shown is the percentage of P-selectin positive platelets (median and interquartile range). Agonist-induced platelet activatability values were corrected for baseline values. $^{*}p < 0.05$, $^{**}p < 0.01$.

Conclusions: The combined results of our study show that NAFLD is not associated with platelet hyperactivity or changes in pivotal proteins in primary haemostasis. The increased cardiovascular risk of these patients thus appears to be primarily related to classical cardiovascular risk factors, and not to liver disease-induced platelet activation.

P1075

IGFBP-1 SIGNIFICANTLY HELPS IN PREDICTING LIVER FAT IN NAFLD

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Background and Aims: Insulin-like growth factor binding protein 1 (IGFBP-1) is a protein exclusively produced by the liver and exclusively regulated by insulin, which might make measurement of this protein an ideal marker for NAFLD. We determined whether measurement of IGFBP-1 helps in non-invasive prediction of liver fat content in NAFLD compared to routinely available clinical and biochemical parameters and genotyping for the PNPLA3 I148M gene variant.

Methods: Liver fat content (proton magnetic resonance spectroscopy, ^{1}H-MRS), clinical and biochemical parameters as well as PNPLA3 genotype at rs738409 were measured in 391 subjects [age 43 (30–54) years, BMI 32.9 (28.1–39.8) kg/m^2, 47% with NAFLD], who were randomly divided into estimation and validation groups. Variables identified by univariate analysis in the estimation group were selected based on Akaike Information Criteria for multiple linear regression analysis. The F test was used to compare the ability of different models to predict liver fat.

Results: In multiple linear regression analysis, the final model i.e. the '% liver fat equation' included age, fasting serum (fS) IGFBP-1, interaction between fS-IGFBP-1 and fS-insulin, fS-triglycerides, fS-ALT and PNPLA3 genotype at rs738409 with an adjusted R of 0.59, $p < 0.0001$. These variables were all independent determinants of liver fat content. The model worked similarly in the estimation and validation cohorts. Exclusion of IGFBP-1 (F-test $p < 0.001$) or both IGFBP1 and the interaction term ($p < 0.0001$) significantly decreased the explanatory power of the model.

Conclusions: These data show that IGFBP-1 helps in the prediction of liver fat content in NAFLD even when routinely available clinical and biochemical parameters and the PNPLA3 genotype is taken into account However, the accuracy remains modest thus perhaps emphasizing the need to develop simple direct measures of liver fat content.

P1076

NON-ALCOHOLIC FATTY LIVER DISEASE IS NOT ASSOCIATED WITH PLASMA HYPERCOAGULABILITY

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is associated with an increased risk of cardiovascular disease; however, it is unclear if subclinical hypercoagulability contributes to this risk. Therefore, we studied the coagulation status in patients with NAFLD.

Methods: We drew blood samples from 68 patients with biopsy-proven NAFLD (simple steatosis n = 24, NASH n = 22, and NASH cirrhosis n = 22), 20 lean controls, 20 overweight controls (BMI >25 kg/m^2), and 15 patients with alcoholic (ASH) cirrhosis. Haemostatic status was assessed by thrombin generation testing, thromboelastography (TEG), and a plasma-based clot lysis assay.

Additionally, plasma levels of proteins involved in clot formation and lysis were measured.
Results: Thrombin generation tested in the presence of thrombomodulin, the endogenous activator of protein C, was comparable between patients and controls. The functionality of the protein C system, however, was decreased in patients with NASH or ASH-related cirrhosis as evidenced by an increase in the ratio between thrombin generation in presence and absence of thrombomodulin. TEG test results were comparable between the lean and overweight controls, patients with simple steatosis, and patients with NASH. However, TEG revealed moderate hypocoagulability in both NASH and ASH cirrhosis as demonstrated by a prolonged K-time and a decreased angle and maximum amplitude. Plasma fibrinolytic potential was comparable between patients with NAFLD and both control groups, but accelerated fibrinolysis was observed in ASH cirrhosis. Both fibrinogen and factor VIII were comparable between all patients and controls. However, protein C and antithrombin were decreased in patients with NASH and ASH cirrhosis. Levels of plasminogen activator inhibitor 1 stepwise increased from healthy controls to patients with simple steatosis, and were still increased in patients with NASH and NASH cirrhosis, but levels were comparable between ASH cirrhosis and healthy controls. In contrast, levels of tissue plasminogen activator were increased in patients with cirrhosis.
Conclusions: The combined results of this study provides no evidence of a hypercoagulable status in patients with NAFLD regardless of the disease stage, which contrasts with previously published results in similar populations. Although the discrepancy between the studies is unclear, our study suggests that the role for haemostasis in the increased risk of thrombosis in patients with NAFLD is limited.

P1077

PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE HAVE SIMILAR GENERAL HEALTH PERCEPTIONS AS THOSE OF HEALTHY CONTROLS

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Background and Aims: Although patients with non-alcoholic fatty liver disease (NAFLD) are generally asymptomatic, they report both physical and emotional lower quality of life and over-utilize medical services as compared to healthy controls. Fatigue and lack of confidence to exercise have been described in NAFLD patients. The study aim was to examine the association between NAFLD and general health perception.
Methods: A cross-sectional study in 213 subjects, with no known liver disease or history of alcohol abuse. The evaluation included: self-reported general health perception, physical activity habits, frequency of physician's visits, fatigue impact scale and abdominal ultrasound.
Results: There were 54% males, mean age 58±9.6 years and 70/213 had NAFLD. All anthropometric measures, liver enzymes and fasting glucose blood levels were significantly higher among NAFLD as compared to controls (age and gender were comparable). Reduced motivation for activity with physical effort (27.1% vs. 33.6%, P = 0.343) and actual need for reduced activity (20% vs. 19.6%, P = 0.781) as well as other parameters in the fatigue impact scale were equivalent between the NAFLD and the control groups. Time spent in leisure time physical activity was lower among the NAFLD subjects, however, fatigue and fear of physical harm as explanations for lack of physical activity were evenly reported between the groups (P = 0.436). Self-reported general health perception status did not differ between NAFLD and control groups. In a multivariate analysis, NAFLD was not associated with a lower self-reported general health perception (OR = 0.735, 95% CI 0.349–1.548, P = 0.417). Nevertheless, the odds for "very good" self-reported general health perception decreased with increasing level of BMI and age, and increased among males and those that exercised regularly. In terms of health service utilization, there was a significant difference in the reasons for family doctor visits (P = 0.016); NAFLD patients had more acute disease visits while controls had more routine checkups and chronic diseases visits.
Conclusions: Patients with NAFLD were comparable to controls in terms of fatigue and self-reported general health perception. Health service utilization due to chronic diseases was less common among the NAFLD group. Our results imply that fatty liver without clinically significant liver disease has a small impact on health perception.

P1078

THE EFFECT OF NON-ALCOHOLIC STEATOHEPATITIS ON ESTIMATED ISCHEMIC CARDIAC RISK

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Background and Aims: Nonalcoholic fatty liver disease has been recognized as a major health burden; patients with nonalcoholic fatty liver disease typically meet the diagnostic criteria for the metabolic syndrome and therefore have multiple risk factors for cardiovascular disease.
Aim: to assess the effect of fatty liver disease on estimated coronary cardiac risk according to the Framingham calculation.
Methods: This prospective study included 150 patients with NASH, and the control group included 120 individuals without a medical history of NASH who were enrolled randomly from the Medicine Clinic the medical staff, The Framingham Heart Study risk equations were used for the calculation of 10-years' estimated cardiovascular risk based on systolic blood pressure, total cholesterol, HDL cholesterol, age, and sex.
Results: Compared with the control group, the NASH group had higher systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides and LDL cholesterol, but lower HDL cholesterol, the median 10-years cardiovascular risk was significantly lower (0.85% in the control group versus 1.72% in the NASH group, p < 0.05). Multivariate analysis showed that HDL-cholesterol <35 (OR = 3.0, 95% CI 2.4–4.1) and serum triglycerides levels >150 (OR = 2.3, 95% CI 2.2–3.1), male sex (OR = 2.2, 95% CI 2.1–3.1), and body mass index >25 (OR = 1.7, 95% CI 1.2–2.1) were associated with height 10-years cardiovascular risk.
Conclusions: 10-years cardiovascular risk among patients with hepatic steatosis is increased. Obesity, low HDL, high triglycerides level, male sex, appear the most predominant factor that influenced the increase 10-years cardiovascular risk.

P1079

PNPLA3 VARIANT p.I148M IMPROVES RESPONSE TO BARIATRIC SURGERY IN OBESE PATIENTS WITH FATTY LIVER DISEASE

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Background and Aims: Obesity is the major trigger of fatty liver disease (FLD). This disorder is further favored by the *PNPLA3* (adiponutrin) p.I148M genotype, which may also predispose to liver cirrhosis and HCC (Krawczyk *et al. Semin Liver Dis* 2013). Currently, bariatric surgery is becoming a more frequent weight loss therapy,

however its effects on liver status with respect to the *PNPLA3* variant remain unknown.
Methods: We prospectively monitored 83 obese individuals (BMI 35–64 kg/m^2) before and after bariatric surgery. The *PNPLA3* p.I148M variant was genotyped using a fluorescent PCR-based assay. All patients underwent liver biopsy at the moment of surgery. Hepatic steatosis was determined before surgery using three different procedures, i.e. (A) Folch: biochemical determination of hepatic triglyceride contents; (B) semiquantitative histological steatosis grade: 0: no steatosis, 1: 1–33%, 2: 34–66%, 3: >66% steatosis; and (C) multi-echo MRI. One year later, steatosis was re-evaluated by MRI and Folch values were estimated with a novel MRI-based equation (Jiménez-Agüero *et al. BMC Med* 2014).
Results: Overall, 54 individuals (65%) carried the *PNPLA3* genotype [II] and 29 (35%) were carriers of at least one prosteatotic allele [M]. The number of individuals carrying this risk allele increased with the grade of steatosis before surgery (grade 0: 25%; 1: 32%; 2: 36%; 3: 42%). Presence of this allele was associated with increased hepatic triglyceride contents (P = 0.01), increased alkaline phosphatase activities (P = 0.01) and a trend to increased MRI-steatosis (P = 0.10). Of note, median weight loss after bariatric surgery was higher in individuals carrying the susceptible *PNPLA3* allele [M] as compared to carriers of genotype [II] (45 *vs.* 37 kg, P < 0.01). Accordingly, patients carrying the prosteatotic allele [M] demonstrated a higher decrease of liver fat one year after surgery as compared to individuals with the common genotype, based on both MRI fat fraction and estimated Folch values (P = 0.05 and P < 0.01, respectively). In regression analysis, the *PNPLA3* mutation outscored weight loss as predictor of FLD improvement (P = 0.05 vs. P = 0.10, respectively).
Conclusions: Obese patients with *PNPLA3*-associated steatohepatitis show better improvement of hepatic steatosis after bariatric surgery as compared to carriers of *PNPLA3* wild-type alleles. Bariatric surgery can reduce the harmful effects conferred by the *PNPLA3* mutation and obesity.

P1080
CHOLECYSTECTOMY IS A RISK FACTOR FOR FATTY LIVER DISEASE
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Background and Aims: Risk factors for fatty liver disease are not well defined, cholecystectomy was considered as a risk factor for fatty liver disease recently. We investigated the association of gallstones and cholecystectomy with fatty liver disease in a regional study.
Methods: Among adult patients admitted in the First Hospital of Jilin University during 2013–2014, gallstone disease and fatty liver disease were diagnosed by abdominal computerized tomography scan or magnetic resonance imaging. Odds ratios (ORs) for the association of gallstone disease with fatty liver disease were calculated using logistic regression analysis to adjust for common associated factors.
Results: Among 59,765 adult patients, the prevalence of gallstones, cholecystectomy and fatty liver disease was 12.7%, 5.4% and 13.0%, respectively. Patients with cholecystectomy had lower age-sex-adjusted prevalence of fatty liver disease (8.6%) than those with gallstones (14.0%) or without gallstone disease (13.2%) ($P < 0.01$ for all comparisons). Controlling for numerous factors associated with both fatty liver disease and gallstone disease, multivariate-adjusted analysis confirmed the association of fatty liver disease with cholecystectomy [OR = 1.452; 95% confidence interval (CI): 1.3–1.7], but not with gallstones (OR = 1.0; 95% CI: 0.9–1.1).
Conclusions: The association of fatty liver disease with gallstone disease indicates that cholecystectomy itself may be a risk factor for fatty liver disease.

P1081
HYPERPLASTIC COLONIC POLYPS LINK TO NONALCOHOLIC STEATOHEPATITIS AND VITAMIN D DEFICIENCY
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Background and Aims: Nonalcoholic steatohepatitis (NASH) is an common disease that link with metabolic syndrome, the prevalence of NASH in Europe and USA range from 14% to 20%. This increase prevalence related directly to obesity epidemic seen in these populations. hyperplastic colon polyp is the most common colon polyp. About 90% of colon polyps are hyperplastic polyps (HP); most of the remaining 10% are adenomas. HP is associated with aging, diet high in fat and beef and low in fiber. Other risk factors are smoking, a lack of exercise resulting in weight gain.
Objectives: To investigate a possible association between NASH and HP.
Methods: The study included 123 patients diagnosed with biopsy proven NASH followed at our outpatient liver clinic during the period March 2010 to May 2013, as well as a control group matched for age and gender (see Table 1). All subjects underwent a complete work-up and medical examination, anthropometric measurements, full colonoscopy and laboratory tests. Laboratory tests included complete blood count, C-reactive protein (CRP), and vitamin D levels.
Results: Mean CRP values were significantly higher in the NASH group (1.1±0.7 mg/dl in the NASH group and 0.4±0.8 mg/dl in the control group, $P < 0.05$). Vitamin D levels were significantly decreased in the NASH group (11.32±10.18 ng/ml in the NASH group vs. 21.55±13.62 ng/ml in the control group, $P < 0.05$). Multivariate analysis showed that CRP (odds ratio 3.1, 95% confidence interval 2.6–4.2, $P = 0.04$) and serum vitamin D levels <30 ng/ml (OR 2.3, 95% CI 2.2–3.1, $P = 0.02$) were associated with NASH.
In the NASH group 14 adenomas were found compared with 16 in the control group (P = NS). HP were more common in the NASH group (28 versus 8 HP in the control group P < 0.05), all the HP found in the NASH group were in the left colon while 2/8 HP in the control group were located at the right colon.
Conclusions: We found a significant correlation between NASH and HP prevalence and vitamin D deficiency. NASH suggested being risk factor to development of HP.

P1082
FATTY ACID OXIDATION CHANGES IN EARLY-ONSET PRE-ECLAMPSIA, HELLP SYNDROME AND ANTIPHOSPHOLIPID SYNDROME AND ITS CORRELATION WITH OXIDATIVE STRESS AND ENDOTHELIAL INJURY IN HUMAN TROPHOBLASTS
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Background and Aims: Preeclampsia syndrome is a multifactorial pregnancy complications which serious threat to maternal-fetal health. We and others have found that abnormal of fatty acid oxidation or lipid metabolisms exist in some cases of preeclampsia syndrome. Especially, there is a relationship between the long-chain fatty acid oxidation disorders and severe pre-eclampsia HELLP syndrome or acute fatty liver of pregnancy. But the correlation between long-chain fatty acid oxidation dysfunction with oxidative stress and endothelial injury in the pathogenesis of preeclampsia is unclear. This study was designed to investigate the effects of

fatty acid oxidation changes in early-onset pre-eclampsia, HELLP syndrome and antiphospholipid syndrome (APS) and its correlation with oxidative stress and endothelial damage in trophoblast cells.
Methods: Primary human trophoblast cells and HTR8/SVneo cells were treated with serum from patients with early-onset severe PE (E-PE group), E-PE with HELLP (PE-HELLP group), APS (APS group) and normal pregnant women served as controls (NC group). Cells treated with various lengths of free fatty acids as long-chain, medium-chain and short-chain were also used as controls. Spearman correlation analysis was performed to evaluate the correlation between serum FFA concentrations, lipid droplet deposition and trophoblast invasion and COX-2, p38MAPK, P47phox and NF-kB protein expression.
Results: Cells in the E-PE group had more severe lipid droplet deposition and showed similar changes with long-chain FFA group. FFA levels in the PE-HELLP group increased significantly compared with the NC group ($P < 0.05$). In the E-PE, PE-HELLP and APS three study groups, COX-2, P38MAPK, P47phox and NF-kB protein expression significantly increased ($P < 0.05$), which were different correlated with FFAs concentration and trophoblast invasion.
Conclusions: The pathological serum from patients had different effects on lipid deposition in trophoblast cells and lead to reduction in trophoblast invasion ability, which was correlated with serum free fatty acid level. Varying degrees of long chain fatty acid oxidation disorders exist in some subset of E-PE, PE-HELLP and APS, which is relate with distinct oxidative stress pathways and different degrees of endothelial injury.
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Alcoholic liver disease and drug induced liver disease

P1083
A NOVEL IN VITRO METHOD FOR INDIVIDUAL CAUSALITY ASSESSMENT OF IDIOSYNCRATIC DRUG-INDUCED LIVER INJURY (DILI)

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Background and Aims: Drug-induced liver injury (DILI) is the major cause for acute liver failure in developed countries and accounts for a significant number of drug withdrawals and restrictions of use. While dose-dependent DILI is not a diagnostic challenge, the diagnosis of idiosyncratic DILI until now relies on exclusion of other causes and identification of the causative agent may be impossible in polymedicated patients [1].
Aim was to investigate whether hepatocyte-like cells derived from peripheral blood by a proprietary method (MetaHeps®) can be used to diagnose or exclude DILI in patients with acute liver injury.
Methods: Clinical data and blood samples were collected prospectively from patients presenting at the Liver Center Munich®, LMU university hospital with acute liver injury and intake of at least one drug with DILI concern. Acute liver injury was defined according to [2] as ALT≥5×ULN or AP≥2×ULN or ALT≥3 and Bilirubin≥2×ULN without history of liver disease or cirrhosis. RUCAM score was used for clinical DILI probability [3]. MetaHeps® were generated as described in [4] and exposed in vitro with the respective drugs. Toxicity was measured by increase in LDH release.
Results: Unequivocal cases of DILI (n = 10) and non DILI causes (n = 8) of acute liver injury were used as positive and negative controls to test the MetaHeps® performance. RUCAM score in DILI cases was 8 (7–10) and 5 (3–6) in non-DILI cases, respectively. MetaHeps® toxicity was 7.5 in DILI cases (0.3–16.1) and 0.7 in non-DILI cases (0.04–1.8), only 1 DILI case was missed by MetaHeps®.
As yet 51 cases have been investigated (31 DILI, 20 non-DILI) showing 94% sensitivity using MetaHeps® or RUCAM. MetaHeps® showed no false positive result (specificity 100%) whereas RUCAM was false positive in 6/20pts (specificity 70%).
ROC-analysis showed superior MetaHeps® performance (AUC 0.97; $p < 0.05$; Figure 1).
Interestingly, MetaHeps® were also able to identify the causative drug in polymedicated DILI patients.

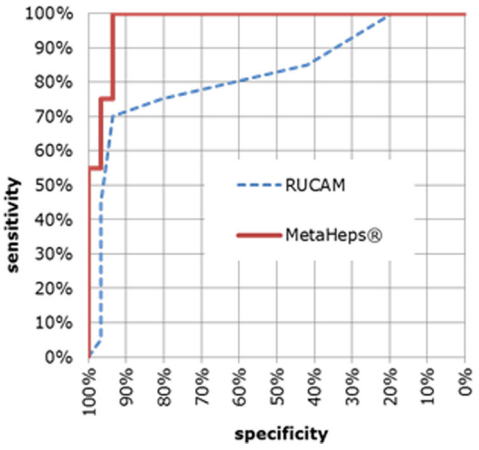

Figure 1.

Conclusions: MetaHeps® are a novel tool for diagnosis of DILI in individual patients allowing also for the identification of the causative agent in polymedication. The remarkable specificity of this promising test could save novel drugs by preventing false positive DILI diagnosis obtained with conventional scores.

Reference(s)

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P1084
COMPOUND FUNCTION OF Jnk1 AND Jnk2 IN HEPATOCYTES IS PROTECTIVE IN ACETAMINOPHEN-INDUCED LIVER INJURY

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Background and Aims: Acetaminophen (APAP)-induced liver injury has been extensively studied since APAP overdose is a leading cause of drug-induced hepatotoxicity (DILI) in Europe and the USA. JNK is strongly activated after APAP-DILI. However, the use of JNK inhibition as a therapeutic approach has considerably failed and new effective treatment options are urgently needed. In the present study, we hypothesized that compound function of *Jnk1* and *Jnk2* is essential for the maintenance of hepatocyte homeostasis during APAP-DILI.

Methods: JNK activation was first evaluated in human biopsies from DILI patients. Female and male mice carrying a compound deletion of *Jnk1* and *Jnk2* in hepatocytes ($JNK^{\Delta hepa}$), RIP-$3^{-/-}$ and control (WT) mice were subjected to APAP *in vivo* and *in vitro*. Additionally, we performed gene array and proteomics analysis in primary isolated hepatocytes and livers.
Results: JNK activation in human DILI was observed in fibrotic areas, infiltrating cells and hepatocytes. Microarray analysis performed in livers and primary hepatocytes (WT compared with $JNK^{\Delta hepa}$) revealing up-regulation of cell death, metabolism, oxidative stress (ROS) and pro-inflammatory related genes in $JNK^{\Delta hepa}$ hepatocytes. $JNK^{\Delta hepa}$–APAP treated mice displayed significantly increased ALT, AST and GLDH, as well as visible severe hepatic haemorrhage. To better characterise the molecular mechanism behind the *in vivo* findings, we next isolated primary hepatocytes from WT and $JNK^{\Delta hepa}$ mice. After 12 h of APAP treatment the morphology of $JNK^{\Delta hepa}$ primary hepatocytes evidenced cytoplasmic projections, loss of cell contact and detachment. Concomitant with these results, we detected a large amount of mitochondrial ROS, TUNEL- and necrotic markers, suggesting a dramatic change of the mitochondrial membrane potential, leading to necrotic cell death, associated with overexpression of RIP-3 and p65 but absence of pJNK and p-c-Jun induction. Since our results indicated that the SP600125-dependent effect in protecting against APAP-induced necrosis could be mediated via RIP-3, we isolated primary hepatocytes from RIP-$3^{-/-}$ mice, which clearly evidenced protection against APAP-induced necrosis.
Conclusions: Compound function *Jnk1* and *Jnk2* in hepatocytes is protective against APAP-DILI. Our results question the current hypothesis that JNK inhibition is a treatment option for APAP-DILI, and thus earlier results using JNK inhibitors overlooked the unspecific inhibition of necrotic cell death.

P1085
MECHANISTIC INSIGHT INTO ACETAMINOPHEN-INDUCED HEPATIC TIGHT JUNCTION DISRUPTION USING A HUMAN HepaRG-BASED LIVERBIOCHIP IMPEDANCE BIOSENSOR

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Background and Aims: Acetaminophen (APAP) hepatotoxicity remains the leading cause of acute liver failure. Intrinsic pathophysiological mechanisms include GSH/ATP depletion, mitochondrial dysfunction, and oxidative stress, culminating in the physical disruption of cellular integrity, and cell death. HepaRG cells are considered surrogates for primary human hepatocytes, with intact Phase I-III drug metabolism/ functional polarity, suitable for APAP toxicity studies. Hepatic tight junctions (TJs) maintain polarity essential for bile secretion, drug transporters and CYP450 expression. Cholestatic drugs, which induce leaky TJs, can generate toxic mechanisms (similar to APAP) in HepaRG cells, including ROS-mediated disruption of TJ-associated F-actin. However, mechanistic insight into APAP-induced TJ disruption in hepatocytes is lacking. Our aim was to investigate dose-dependent effects of APAP on TJs in HepaRG cells using a non-invasive/real-time impedance-based biosensor array (LiverBioChip), correlated with biochemical and morphological phenotypic assays.
Methods: Differentiated HepaRGs were cultured to confluence on 96-well (96-idf20) electrode arrays. On day 8, following 24 h Rifampicin-CYP3A4 induction, APAP time-/dose-response [0–20mM] was monitored with quantitative impedance, |Z|,

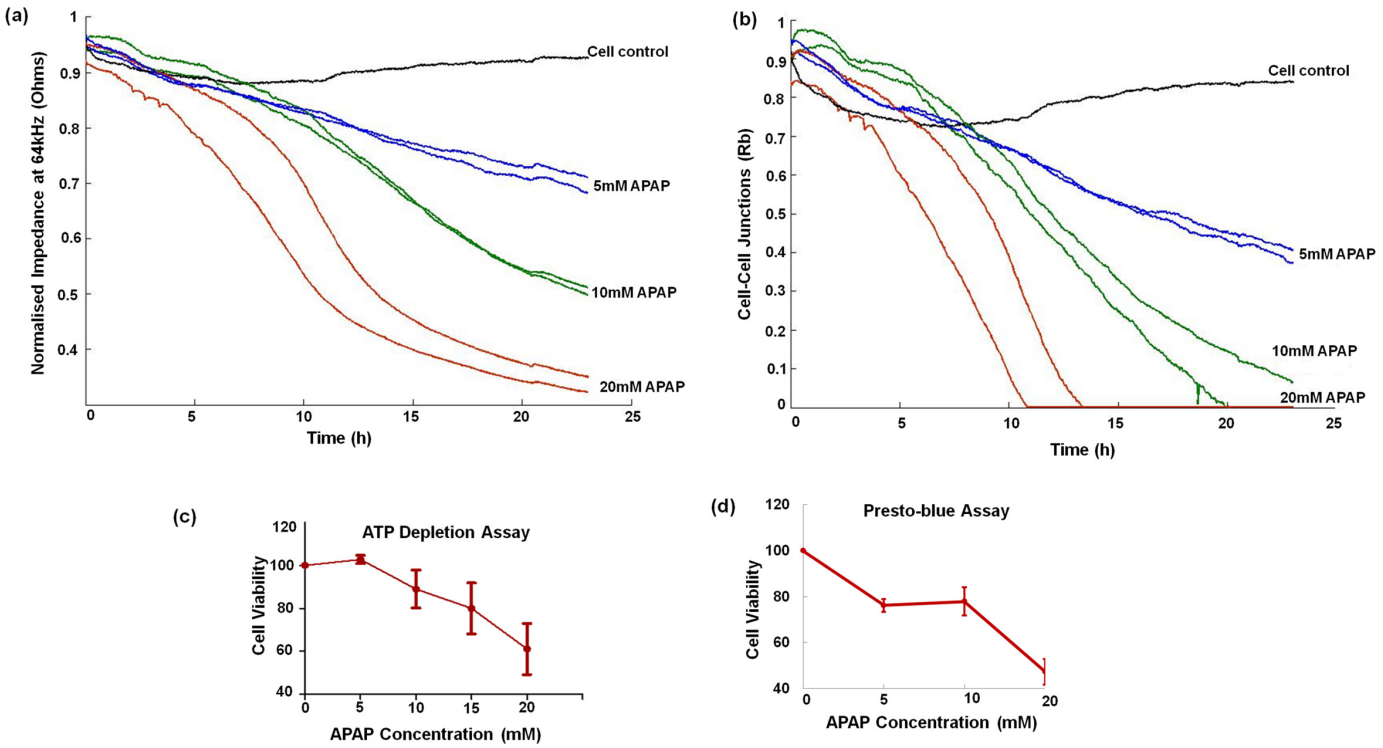

Figure 1 (abstract P1085). Acetaminophen challenge. Dose-dependent effects on: (a) Total impedance (cell behavior index); (b) Tight junction changes (Rb) showing early dose-dependent disruption of Tjs, even at sub-toxic APAP levels (5mM). Impedance data correlated with endpoint hepatotoxicity. (c) ATP depletion, and (d) Prestoblue live-cell viability assays at 24 hours.

measurements (180s intervals; f = 4 kHz) for 96 h post-induction; to detect the TJ parameter (Rb), using |Z|-spectra modeling. Correlative hepatotoxicity/phenotypic assays were performed: (i) ATP-depletion; Prestoblue (PB: live-cell viability); (ii) Phorbol Ester (disrupts TJ integrity); (iii) Expression of TJs: ZO-1; TEM-Ultrastructure; (iv) M30/M65 apoptosis/necrosis cell death kits.
Results: Real-time |Z| monitoring showed highly-sensitive/temporal dose-response to APAP, whilst subsequent |Z|-spectra modelling reflected early disruption of TJs (Figure 1). Endpoint ATP-depletion and PB assays correlated with |Z| changes (24 h). Ultrastructural imaging coupled with ZO-1 confirmed disruption to TJ-barrier function, prior to cell death (type 2 blebbing). M30/M65 assays showed apoptosis (M30) as a pre-dominant early event (measured at 12 h) in response to APAP >10mM, as TJ integrity detected by impedance (Rb) fell to 50% of controls.
Conclusions: LiverBioChip provides a continuous/quantitative real-time indicator of hepatic TJ integrity; revealing early, dose-dependent disruption of TJs even at sub-toxic APAP levels (5mM). This platform may provide mechanistic insight into hitherto unknown effects of APAP, including mode of cell death.

P1086
COMPLEX EFFECTS OF ADENOSINE RECEPTOR ANTAGONISTS IN MODELS OF LIVER FIBROSIS

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Background and Aims: There are four known subtypes of adenosine receptors (AR; namely A_1, A_{2A}, A_{2B} and A_3), but the role of ARs in fibrosis is not fully explored. We investigated the antifibrotic effect of a spectrum of subtype specific AR antagonists in liver fibrosis.
Methods: The AR antagonists: DPCPX for A_1R; ZM241385, 8-(3-Chlorostyryl) caffeine, SCH-58261, and KW6002 for A_{2a}R; Enprofylline, Xanthine amine congener (XAC), and Alloxazine for A_{2b}R; MRS1523 for A_3R were tested in two murine liver fibrosis models. Six week old female C57BL/6 mice were orally gavaged with increasing doses of CCl_4 for 2 weeks (n = 5/group) and received a low (0.5 mg/kg/d) or high dose i.p. (2 mg/kg/d) of antagonists in the second week. Mdr2KO mice received the antagonists from age 6 to 10 weeks of age (n = 6/group). Vehicle was used for control groups. Mice were analyzed for liver collagen (hydroxyproline, Sirius red morphometry), serum biochemistry (AST, ALT, ALP, creatinine, total bilirubin), and qPCR for fibrosis related transcripts [procollagen α1(I), α-SMA, TGFβ1, integrin β6, and TIMP-1]. The intrahepatic cAMP levels of A_2R antagonists treated mice were measured.
Results: The A_1R antagonist DPCPX did not show any significant effect on fibrosis progression. Among the A_{2a}R antagonists, the most specific and active drug, SCH-58261, modestly aggravated fibrosis in both models at the low dose. Among the A_{2b}R antagonists, low dose Alloxazine significantly attenuated collagen accumulation, decreased procollagen α1(I) and integrin β6 mRNA expression, but upregulated TGFβ1 transcripts at the high dosein both models. Enprofylline (equipotent for both A1 and A2R) and XAC tended to increase fibrosis at all doses in both models, possibly due to their lower specificities compared to Alloxazine. Finally, the A_3R antagonist MRS1523 significantly aggravated all fibrosis related parameters at the low dose. Effective antagonism of adenosine R antagonists was demonstrated by suppression cAMP levels.
Conclusions: We demonstrated an antifibrotic effect of A_{2b}R and A_3R inhibition, and a profibrotic effect of A_{2a}R inhibition. In some instances divergent dose-dependent anti-fibrotic effects of adenosine receptor antagonists may be due to overlapping specificities in some cases, a narrow therapeutic window, and to different cell types addressed at variant doses.

P1087
PREDICTION OF DEATH AS A CONTINUUM OF RISK OF MORTALITY IN SEVERE ALCOHOLIC HEPATITIS BASED ON BASELINE AND DYNAMIC MODELS

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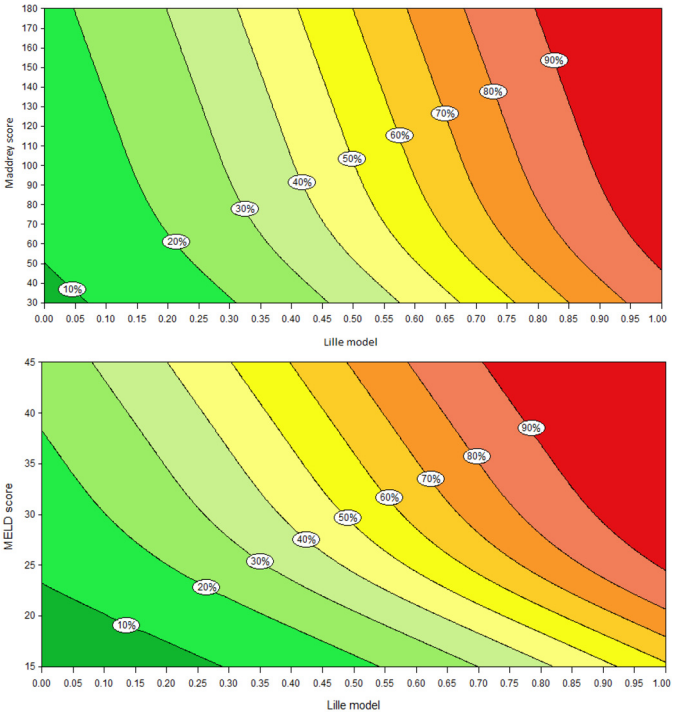
Background and Aims: Several scoring systems have shown their accuracy to predict survival of patients with severe alcoholic hepatitis (AH) but their use is mainly based on one or two cut-offs. This assessment is based on pre-treatment measures of disease severity for static models (MELD score, Maddrey discriminant function DF) but also on-treatment models which assess early improvement in liver function (Lille model). Combining information from these two types of models may help in assessing outcome of patients as a continuum of probabilities of dying.
Methods: Based on a large international database of patients with severe AH (Maddrey DF≥32) treated with corticosteroids, we combined information from static models (Maddrey DF or MELD score) and from the Lille model. Contour lines were built to assess the probability of death at 6 months.



Results: 897 patients were included: age 50.6 years, 58.5% of males, bilirubin 156 μmol/l, prothrombin time 20.2 s, creatinin 8 mg/l, DF 55, MELD 25.2, Lille model 0.34. Six-month survival was 64.1%.

Figure (top) gives the probability of death using DF at day 0 (Y-axis) and Lille model at day 7 (X-axis). As an example, a patient admitted with a DF at 100 with a Lille model at day 7 of 0.25 has a probability of death of 27% at 6 months, which increases at 72.3% if Lille model is 0.7. The likelihood of the joint-effect model was improved in comparison to a model based solely on Lille model ($p = 0.016$). We conducted a second analysis Lille-MELD on a subgroup of 638 patients for whom MELD score was available (Figure, bottom). The efficacy of the joint-effect model was also better as compared to each model alone ($p < 0.001$). As an example, a patient admitted with a MELD score at 28 with a Lille model of 0.25 has a probability of dying of 27% at 6 months, which increases at 69.7% if Lille model is 0.7. The two combinations (Lille+Maddrey DF and Lille+MELD) showed good discrimination and calibration, the latter meaning that predicted survival fitted the observed survival. Using the Akaike information criterion, we found that the combination Lille+MELD was a better fitting prognostic model as compared to the combination Lille+Maddrey DF.

Conclusions: We propose a new approach that predicts the outcome of patients with severe alcoholic hepatitis in terms of a continuum risk of mortality. This may be helpful not only to improve management of patients but also to develop trials for future strategies.

P1088

MORPHOMETRICAL QUANTIFICATION OF FIBROSIS CORRELATES WITH CLINICAL CIRRHOSIS STAGE AND PREDICTS LONG-TERM SURVIVAL IN PATIENTS WITH ALCOHOLIC CIRRHOSIS

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Background and Aims: Histological subclassification of cirrhosis may allow for the definition of severe fibrosis stages with different clinical severity and prognosis. Recently the Laennec fibrosis scoring system was found to correlate with the clinical cirrhosis stage and grade of portal hypertension. However, the value of histological staging is limited by intra- and interobserver variability. We therefore aimed to determine the relationship between morphometrically quantified fibrosis measured as collagen proportionate area (CPA) using digital assisted image analysis and clinical stage of septal fibrosis and cirrhosis as well as long-term survival in patients with alcoholic liver disease (ALD).

Methods: Liver biopsies of 149 consecutive ALD-patients, without other cause of liver disease were studied. CPA was quantified using Tissue Studio ® software (Definiens™). The data were externally validated at the National University of Singapore. In addition fibrosis was staged semiquantitively using the clinical research network (CRN) and Laennec systems. Clinical stages at the the time of liver biopsy were determined according to D'Amico. Complete follow up and outcome data were available from all patients.

Results: Mean CPA values progressively increased with Laennec fibrosis stage (stage 0: 1.4±0.8%, stage 1: 3.5±2.6%, stage 2: 8.8±6.1%, stage 3: 14.6±9.2%, stage 4: 21.1±11.8%, stage 5: 34.8±14.0%, stage 6: 48.1±12.4%; $p = 0.000$ by ANOVA) and with clinical D'Amico stage (stage 1: 12.5±13.7%, stage 2: 30.0±19.8%, stage 3: 40.2±18.2%, stage 4: 35.7±18.2%; $p = 0.000$ by ANOVA). On ROC analysis, a CPA of 35% was identified as best cut-off for discrimination of cirrhosis. In patients with septal fibrosis or cirrhosis (CRN stage >2), Kaplan–Meier analysis revealed an association of 5-year survival to CPA (cut-off 35%, $p = 0.035$) but not to Laennec fibrosis stage ($p = 0.294$).

Conclusions: These findings suggest that morphometrical subclassification yields two fibrosis stages with distinct clinical outcomes. CPA measurements may favorably substitute for semiquantitative histological fibrosis staging used in ALD while avoiding intra- and interobserver biases which limit the utility of conventional histological fibrosis staging.

P1089

CELLULAR SENESCENCE AND LIPOPOLYSACCHARIDE/TOLL-LIKE RECEPTOR 4 SIGNALING IN ALCOHOLIC STEATOHEPATITIS

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Background and Aims: Cellular senescence is a stress-responsive program limiting the proliferation of damaged cells and leading to stable cell-cycle arrest. Accumulation of senescent hepatocytes may contribute to loss of functional hepatic mass and lead to liver decompensation and fibrosis. The current study aimed to characterize the functional role of cellular senescence during alcoholic-induced hepatitis.

Methods: Senescence related gene expression was assessed using a Cellular Senescence PCR Array and/or real-time PCR analysis. Cellular senescence and viability were measured by SA-β-gal Activity and MTS assay. The upstream modulators of senescence were defined in ethanol/LPS treated hepatocytes and cholangiocytes in vitro, and in ethanol fed mice and TLR-4 knockout mice in vivo.

Results: We identified that 5 week of ethanol feeding significantly increased total liver histopathology score and hepatocellular senescence by PCR array and SA-β-gal assay. The up-regulation of hepatic senescence initiators, PAI-1 and EGR1, were further verified by real-time PCR assay. Treatment of N-Heps and HiBECs with ethanol (86 mM) and LPS (20 ng/ml) for 72 hr significantly increased PAI-1 and EGR1 expression, along with the enhanced SA-β-gal activity. Silencing of PAI-1 decreased *ethanol and LPS*-induced *senescence* in both N-Heps and HiBECs, whereas inhibition of EGR1 only reduced the senescence and increased viability in N-Hep cells. Interestingly, silencing of PAI-1 and EGR1 also reduced the pro-fibrotic markers, α-SMA and TIMP-1 in N-Hep cells, whereas silencing of LPS receptor TLR4 decreased these markers in the same group of N-Hep cells, suggesting LPS-mediated cellular senescence during alcohol-induced liver fibrosis. Furthermore, the expression of TLR4 and the verified LPS related senescence markers, including E2F1, ID1 and IGFBP3 were significantly altered in ethanol-fed mice liver specimens compared to controls. TLR4 knockout mice displayed less sensitivity to alcoholic injury, along with reduced PAI-1 and EGR1 levels and recovered expressions of α-SMA and TIMP-1.

Conclusions: Our results show that PAI-1 and EGR1 are the critical regulator of LPS induced cellular senescence and alcoholic hepatitis. The findings provide new insight into the function of LPS regulated cellular senescence and increase opportunities for the development of novel treatment paradigms for the management of alcoholic liver diseases.

P1091

ONE YEAR EFFECTIVENESS OF BACLOFEN TREATMENT IN 100 ALCOHOL-DEPENDENT PATIENTS

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Background and Aims: Several studies have suggested efficacy of Baclofen (BAC) at low or high dose in reducing alcohol consumption. Since March 2014, Temporary Recommendation for Use of BAC has

been allowed by the French drug agency (ANSM) in this indication. The aim of the study was to assess effectiveness and safety of BAC at 12 months in alcohol-dependent patients with or without liver cirrhosis.

Methods: Between June 2010 and September 2013, 100 consecutive patients from 2 liver and alcohology units were included in this prospective open label study. Patients provided written consent before treatment initiation. BAC was orally administered at a dose of 15 mg/day and weekly increased until alcohol indifference was obtained. The treatment was associated to social-psychological support and medical care.

Results: BAC was started in 100 patients (75 males, mean age 53±9 years): 65 were cirrhotic and 16 had a chronic pancreatitis. After 1 year, 86 patients were still involved in the follow up, 83 were treated with BAC, 9 were lost of follow-up, 4 were dead and 1 had been transplanted. At a mean BAC dosage of 40 mg/day [30–210], mean daily alcohol consumption (DAC) was reduced from 106 to 18 g/day ($p < 0.001$). A decrease of the DAC >50% was observed in 77 patients. Among them, a "low consumption group" of 64 patients was identified: 44 were completely abstinent and 20 drunk less than 30 g/day. No predictive factor of response was identified. In this group, a significant improvement of consumption biomarkers was observed: decrease of mean γGT activity from 4.8N to 2N ($p < 0.001$), mean ASAT activity from 2.6N to 1.1N ($p < 0.001$) and mean erythrocyte globular volume from 100.6 to 92.8 μ^3 and increase of mean platelets count from 171000 to 193000/mm^3 ($p = 0.032$). In the 39 cirrhotic patients of the "low consumption group", total bilirubin serum concentration significantly decreased from 34.2 to 19.5 μmol/L ($p = 0.026$), prothrombin time increased from 69 to 77% ($p < 0.001$) and albuminemia increased from 34.2 to 37.2 g/L ($p = 0.07$). Twenty patients (20%) reported minor side effects leading to a treatment withdrawal in 2 cases. No liver or renal function deterioration occurred in cirrhotic patients.

Conclusions: In our cohort, baclofen treatment associated to a global care led to a dramatic reduction of alcohol consumption. This effective treatment is well tolerated and associated with a significant improvement of consumption biomarkers and of liver function tests in cirrhotic patients.

P1092

SPECIFIC-SIZED HYALURONAN FRAGMENTS DIFFERENTIALLY REGULATE LPS SIGNAL TRANSDUCTION IN KUPFFER CELLS AFTER ETHANOL FEEDING TO RATS

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Background and Aims: Hyaluronan (HA), a major component of extracellular matrix, is ubiquitously present in many tissues. During acute and chronic inflammation or tissue injury, reactive oxygen species and matrix metalloproteinases increase HA turnover, resulting in the local and systemic accumulation of HA fragments of different molecular weights. Depending on their size, HA fragments can have either anti-inflammatory, anti-angiogenic and immunosuppressive function or pro-inflammatory function. HA is recognized as an important damage associated pattern molecule (DAMP) that regulates innate immunity. HA has been used as an indicator of liver injury for decades; however, it is not known if HA contributes to the pathophysiology of chronic ethanol-induced liver injury. Chronic alcohol consumption is associated with an increase in the sensitivity of macrophages to signaling via TLR4.

Here we have tested the hypothesis that specific-sized HA fragments differentially regulate LPS-mediated signaling via TLR4 in Kupffer cells, the resident macrophage in the liver.

Methods: Primary cultures of Kupffer cells were isolated from rats chronically exposed to ethanol via the Lieber-DeCarli diet or pair-fed control diets.

Results: Kupffer cells from ethanol-fed rats were more sensitive to stimulation with LPS, resulting in increased expression of mRNA for the pro-inflammatory cytokine, TNFα. Challenge of Kupffer cells with specific-sized HA fragments (from 7 kDa to 2000 kDa) had no effect on TNFα mRNA expression. However, when Kupffer cells were pre-treated with HA fragments for 5 h prior to LPS challenge for 60 min, specific-sized HA fragments differentially suppressed or stimulated TNFα mRNA expression. When Kupffer cells were pre-treated with HA fragments of 35kDa, the sensitization of Kupffer cells from ethanol-fed rats to LPS returned to pair-fed values. In contrast, pre-treatment with 74kDa HA had a synergistic effect in production of TNFα, increasing expression in Kupffer cells from both ethanol- and pair-fed rats. HA fragments, of 7kDa or 2000kDa, had no effect on TNFα production.

Conclusions: In summary, these data suggest that specific-sized HA fragments differentially regulate TLR4-mediated signaling in Kupffer cells and that HA 35kDa fragments may play a protective role in alcoholic liver disease by decreasing the production of inflammatory mediators by Kupffer cells.

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P1093

CHARACTERIZATION OF ACETAMINOPHEN THERAPEUTIC MISADVENTURE: A SINGLE CENTRE EXPERIENCE

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Background and Aims: Therapeutic doses of acetaminophen may provoke acute liver failure, the so-called "therapeutic misadventure with acetaminophen" but this entity has never been evaluated with a prospective design. Such study would help determining prerequisites of this condition and assessing outcome.

Methods: Single-centre prospective study in which patients were included with severe acute liver failure related to acetaminophen as follows: acetaminophen overdose (AO) or intake at a therapeutic dose (≤6 g/day), referred to as acetaminophen therapeutic misadventure (ATM). Chronic drinkers were defined by an alcohol consumption ≥30 g/day.

Results: From 2002 to 2014, 271 patients were included, 205 AO and 66 ATM. ATM occurred only in chronic drinkers (89.4%) or in patients who had starved for several days (10.6%). By definition, acetaminophen intake was higher in AO as compared to ATM: 16 g vs. 3.15 g ($p < 0.001$). In 70% of patients, intake was below the daily 4 g dose classically regarded as safe. No patient developed ATM after a single intake of acetaminophen as confirmed by a median time of intake at 4 days vs. 1 in AO ($p < 0.001$). At admission, patients with ATM were older than AO (age 44 vs. 30.1 years; $p < 0.001$) and had a deeper impairment of liver function (prothrombin time 30.7 vs. 22.1 s, $p < 0.01$; albumin 32.7 vs. 38.1 g/l, $p < 0.001$; bilirubin 47.1 vs. 25 mg/l, $p < 0.001$; lactate 3.31 vs. 1.95 mmol/l, $p = 0.009$). At admission, transaminases were not different between ATM and AO: AST 4138 vs. 3983 IU/l ($p = 0.1$), ALT 2416 vs. 3831 IU/l ($p = 0.22$). In ATM and AO, we found a predominance of females: 63.6% and 56.6% ($p = 0.31$) and percentage of chronic drinkers was 89.4 vs. 36.6% ($p < 0.001$), respectively. As expected in acetaminophen poisoning, we observed a dramatic drop after 3 days by 67% vs. 50.2% ($p < 0.001$) in ALT and by 93.6 vs. 89.4% ($p = 0.02$) in AST in ATM and AO respectively. One-month overall survival was 84.6% in ATM and 92.6% in AO ($p = 0.05$). On the global cohort, only the number of King's College criteria (risk ratio 3.15, 95% CI: 2.04–4.88, $p < 0.001$) independently predicted 1-month mortality, whereas albumin, age and alcohol consumption did not ($p = 0.13$, 0.41 and 0.75 respectively).

Conclusions: Therapeutic misadventure with acetaminophen is not a rare condition and occurs after several days of acetaminophen

intake at a dose <6 g/day, mainly in chronic alcohol drinkers. Its outcome is worse as compared to acetaminophen overdose. Physicians must be aware of the risk of therapeutic doses of acetaminophen in chronic drinkers.

P1094

HIGH LONG-TERM MORTALITY IN DECOMPENSATED ALCOHOLIC LIVER DISEASE (ALD) INCLUDES AN EXCESS OF NON LIVER-RELATED DEATHS

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Background and Aims: Long-term mortality in ALD is not well characterised. We have previously (Al-Joudeh, J Hepatol 2013 58: S211) reported high long-term mortality in 249 patients [162 men, mean age (range) 50 (27–87) years] admitted consecutively to our unit between 1/4/1998 and 31/12/2005 with first presentation of decompensated ALD (Child grade B or C). Here, we aimed to assess further the causes of death in this cohort.
Methods: We reviewed hospital records, and death certificates and contacted surviving patients and GPs to assess who had died, the causes of death and the patients' overall alcohol-drinking behaviour subsequent to the index hospital admission (classified as: abstinent, continued drinking but reduced and did not reduce). We collected data on cardiovascular risk factors from hospital records.
Results: Median (range) follow up was 3.6 (0–16) years. By 1/7/2014, 44 of the 249 patients remained alive (including one of only 2 patients transplanted), 6 were lost to follow up and 199 had died (including the other transplanted patient). 154 deaths were due to complications of liver disease (only 5 due to hepatocellular carcinoma) and 45 to non-liver causes (16 extra-hepatic malignancy, 13 cardiovascular disease, 7 cerebral haemorrhage and 9 other causes). Mortality from non-liver causes was (mean±SEM) 10±3% and 30±5% after 5 and 10 years respectively. Standard Mortality Ratio (compared to the regional population) was 16.0 (95% CI 13.7–18.2) for all-cause death and was 3.6 (2.6–4.7) if only non-liver deaths were considered and cases censored following liver-related death (or transplant). The estimated excess of deaths from non-liver causes (32) exceeded the number of all-cause deaths within the first 30 days (27). Predictors of liver-related death were baseline prognostic scores (Maddrey, Glasgow, MELD and Child), but not for deaths after first four months of follow up; also, serum albumin on discharge from ($p = 0.006$) and drinking behaviour subsequent to ($p < 0.001$) index admission. In contrast, non-liver death showed no association with these variables, or with smoking status, presence of arterial disease, diabetes, treatment for hypertension or hyperlipidaemia or social deprivation index. Non-liver related death was significantly associated with age ($p = 0.012$).
Conclusions: Patients with first presentation of decompensated ALD have high long-term mortality, including previously undocumented excess mortality unrelated to liver disease.

P1095

ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN A MODEL OF CHRONIC-BINGE ETHANOL EXPOSURE IN MICE

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Background and Aims: The innate immune system is a critical factor in the development and progression of Alcoholic Liver Disease (ALD) in both human and animal studies. Macrophage Migration Inhibitory Factor (MIF) is an important innate immune regulator previously identified as a key contributor to liver injury in mice after chronic ethanol feeding. MIF is a pleiotropic protein with cytokine and chemokine properties and may therefore mediate liver injury directly and/or indirectly via immune cell recruitment. While chronic ethanol exposure results in steatosis and modest inflammation in the liver, it does not model more acute, severe alcoholic hepatitis. Here, we have tested the hypothesis that MIF also contributes to liver injury in response to the chronic-binge model of ethanol exposure in mice that more closely models more severe alcoholic hepatitis.
Methods: Wild-type C57Bl/6J and $MIF^{-/-}$ female mice were fed a liquid diet of ethanol for 10 days (5% v/v) then acutely binged with ethanol (5 g/kg) on day 11.
Results: After chronic-binge ethanol exposure, WT mice had increased liver damage compared to pair-fed mice, as assessed by ALT and AST activities. Surprisingly, ALT and AST activities were higher in $MIF^{-/-}$ mice versus WT mice after chronic-binge, suggesting that MIF protects from the acute inflammatory response elicited by chronic-binge ethanol exposure, contrasting with the disease-promoting role of MIF during chronic ethanol exposure. $MIF^{-/-,}$ but not WT mice, had increased TNF mRNA in response to the ethanol, which further implicated MIF as protective in a model of chronic-binge ethanol exposure. $MIF^{-/-}$ mice, however, were protected from excessive triglyceride accumulation in the liver, suggesting that ethanol-induced steatosis is MIF-dependent. In contrast, chronic-binge increased IL-1β, Ly6G and CD11b expression in both WT and $MIF^{-/-}$ livers, which indicated immune cell-recruitment and inflammasome activation were each MIF-independent responses.
Conclusions: Taken together, these results indicate that MIF differentially contributes to liver injury in the Chronic-Binge model, protecting from inflammation and hepatocyte injury, while contributing to steatosis.
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P1096

MIXED LINEAGE PROTEIN KINASE 3 CONTRIBUTES TO ETHANOL-INDUCED LIVER INJURY

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Background and Aims: The progression of alcoholic liver disease (ALD) is associated with death of hepatocytes by both apoptosis and necrosis/necroptosis. Specific pathways of cell death may influence progression of ALD, with hepatocyte death by apoptosis driving fibrotic responses, while death by necrosis/necroptosis and release of DAMPs leading to sterile inflammatory responses. Understanding the pathways regulating apoptosis vs. necrosis/necroptosis after chronic ethanol exposure is critical to the design of appropriate therapeutic interventions for each stage of ALD. We have recently reported that caspase-independent programmed cell death, termed necroptosis, is a primary driver of hepatocyte cell death after chronic ethanol exposure. Since activation of c-jun N-terminal kinase (JNK) contributes to receptor interacting protein 3 (RIP3)-mediated necroptosis and mixed lineage kinase 3 (MLK3) contributes to JNK activation, here we tested the hypothesis that MLK3-dependent JNK activation is critical for ethanol-induced liver injury.
Methods: Female C57BL/6 (wild-type) and MLK3-deficient mice were chronically exposed to ethanol via the Lieber-DeCarli diet.
Results: In wild-type mice, chronic ethanol feeding increased plasma ALT/AST, a marker of hepatocyte injury, hepatic triglyceride content and expression of pro-inflammatory cytokines. Ethanol-induced hepatocyte injury was associated with increased expression of RIP3 protein, an indicator of necroptotic cell death, as well as the accumulation of both TUNEL-positive cells and

M30/CK18-cleavage staining, indicators of hepatocyte apoptosis. Indicators of oxidative stress, including the accumulation of 4-HNE-protein adducts and JNK phosphorylation, were also increased. In contrast, mice deficient in MLK3 were protected from ethanol-induced increases in plasma ALT/AST, pro-inflammatory cytokines and hepatic protein expression of RIP3. Ethanol-induced JNK phosphorylation and oxidative stress were also attenuated in MLK3-deficient mice. However, MLK3-deficiency did not affect ethanol-induced steatosis or hepatocyte apoptosis.
Conclusions: Taken together, these results suggest that MLK3 participates in the development of ethanol-induced oxidative stress, activation of JNK and induction of necroptotic programmed hepatocyte death. Pharmacological intervention of this pathway could be targeted as a potential therapeutic strategy to suppress necroptosis-induced inflammation and hepatocyte injury in patients with ALD.

P1097
DISTINGUISHING DRUG INDUCED AUTOIMMUNE HEPATITIS FROM IDIOPATIC AUTOIMMUNE HEPATITIS

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Background and Aims: Drug-induced autoinmune hepatitis (DI-AIH) is a particular clinical phenotype of hepatotoxicity, poorly defined. Its prevalence in cohorts of patients with DILI is unknown. We aimed to characterize phenotypes and used drugs in DI-AIH, from a large cohort of DILI patients, and in idiopathic autoimmune hepatitis (AIH) to improve differential diagnosis between both entities.
Methods: Demographic and clinical variables in 21 patients out of 1013 included in the Spanish-Latin DILI Registry presenting with typical AIH clinical and analytical features including serum detectable titers of ANA and/or ASMA, as well as high gammaglobulin levels after the initiation of different drug therapies were compared with 51 patients diagnosed with idiopathic AIH at Mendaro and Mondragon Hospitals, Gipuzkoa, Spain.
Results: Both cohorts demonstrated similar age and gender distribution, with 38% males (mean age 58 years ±16) in the DI-AIH cohort. Hypertension and diabetes mellitus were more frequent in DI-AIH patients, 33% vs 12% ($p = 0.023$) and 14% vs 2% ($p = 0.038$), respectively. Other underlying autoimmune diseases were less frequent in DI-AIH (24% vs 33%). The more frequent drugs triggering DI-AIH and the co-medications in AIH patients were statins (19% vs 12%), NSAIDs (10% vs 12%), antibiotics (24% vs 2%) and anti-TNF (10% vs 2%). At presentation patients with DI-AIH exhibited jaundice in the 62% vs 31%, higher total bilirubin levels [6 times the upper limit of normal (×ULN) vs 2×ULN, $p = 0.001$], AST (23×ULN vs 11×ULN, $p = 0.001$), ALT (27×ULN vs 14×ULN, $p = 0.001$) and GGT (9×ULN vs 5×ULN, $p = 0.02$). No significant differences were observed in ALP values. Advanced fibrosis (F3–F4) in liver biopsy was observed in 18% of DI-AIH vs 25.5%. The DI-AIH group required steroid therapy less frequently than the AIH group (81% vs 93%) achieving complete response in 100% of cases vs 92% in AIH.
Conclusions: DI-AIH is more severe than idiopathic AIH at diagnosis, presenting most frequently with jaundice, but liver histology demonstrating a minor grade of fibrosis. Statins are increasingly being identified as potential triggers for DI-AIH and could be actually the unidentified trigger behind many cases of "true idiopathic" AIH.
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P1098
INFLUENCE OF METABOLIC RISK FACTORS IN HEPATOTOXICITY (DILI) PHENOTYPE AND OUTCOME

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Background and Aims: It has been suggested that metabolic risk factors may play a role in DILI presentation. Hence, the aim of this study was to determine the influence of diabetes and dyslipidemia in the clinical profile of DILI.
Methods: 864 DILI cases included in the Spanish DILI Registry were compared according to the presence or absence of diabetes and dyslipidemia.
Results: DILI patients with (n = 121) dyslipidemia were significantly older than those without (n = 743), 64 vs 52 years, ($p < 0.001$) and had on average a higher BMI, 27 vs 25 ($p = 0.017$). Duration of treatment of the causal agent and time to DILI onset did not differ significantly between the groups. In terms of DILI severity, only four severe and fatal cases (3%) were present among the patients with dyslipidemia, while 95 (13%) were found among normolipemic patients ($p = 0.009$). Persistent liver damage more than one year after DILI onset was seen in 30% of the patients with dyslipidemia and 22% of non-dyslipidemia patients. The mean value of total bilirubin at onset was lower in patients with dyslipidemia (5.4 vs 7 ×ULN, $p = 0.018$).
Comparing diabetic (n = 103) with non-diabetic (n = 737) DILI patients, higher mean age (66 vs 52, $p < 0.001$) and BMI (28 vs 25, $p < 0.001$) were observed in the former group. Similarly, duration of treatment of the DILI causal agent and time to DILI onset were longer in the diabetic group, 158 vs 78 days ($p = 0.001$) and 140 vs 71 days ($p = 0.002$), respectively. No differences were found with regards to DILI severity. In the diabetic group 28% of the cases had persistent hepatic injury after one year from DILI onset versus 22% in the non-diabetic group.
In patients with both diabetes and dyslipidemia (n = 33), duration of treatment of the causal agent and time to DILI onset were both

significantly longer compared to patients with none or only one of the conditions (n = 831), 162 vs 84 days ($p = 0.05$) and 157 vs 76 days ($p = 0.037$), respectively. Forty percent of the patients with diabetes and dyslipidemia demonstrated persistent hepatic damage more than year from DILI onset compared to 22% in those without these cardiovascular risk factors.

Conclusions: Patients with dyslipidemia manifested less severe forms of DILI, while diabetic patients required longer treatment time prior to DILI initiation. Persistent damage more than one year from DILI onset was higher in patients with both dyslipidemia and diabetes.

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P1099

SARCOPENIA AS A PROGNOSTIC FACTOR IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS

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Background and Aims: Sarcopenia has emerged as an independent predictor of clinical outcomes in a variety of clinical conditions. The aim of this study was to examine the association between the sarcopenia and the early mortality (90-days) or overall survival in the patients with severe alcoholic hepatitis (SAH).

Methods: Eighty-one consecutive patients with SAH [Maddrey's discriminant function (DF) ≥32] were retrospectively analyzed. Skeletal muscle cross sectional area was measured on a computed tomography (CT) image at the L3 level, and sarcopenia was defined using previously established cutpoints

Results: Sixty-six patients were male (81.5%), and mean age was 49.6±9.8 years with median follow-up of 7.4 months. Overall 90-day mortality was 30.9% and 55 patients (67.9%) had sarcopenia. There were no significant differences in baseline characteristics between patients with sarcopenia and without sarcopenia except high Glasgow Alcoholic Hepatitis Score (GAHS) in sarcopenic group (8.73±1.25 vs 8.23±1.24). By univariate logistic regression analysis, presence of infection (OR, 2.47; $P = 0.065$), hepatic encephalopathy (HE) (OR, 9.00; $P < 0.001$), spleen size (OR, 0.77; $P = 0.036$), INR (OR, 7.89; $P = 0.002$), serum creatinine (OR, 1.73; $P = 0.014$), and leukocyte count (HR, 1.03; $P = 0.037$) appeared to be potential risk factors of short-term mortality ($P < 0.1$). However, sarcopenia was not associated with 90-day mortality (OR, 2.40; $P = 0.125$). By multivariate logistic regression analysis, INR (OR, 6.92; $P = 0.016$) and HE (OR, 6.13; $P = 0.018$) were independently associated with 90-days mortality. Although not statistically significant, sarcopenia tended to have association with HE (OR, 2.90; $P = 0.082$) at the time of admission. By Kaplan–Meier method, sarcopenic group had shorter overall survival time (47.7 vs. 29.3 months, $P = 0.072$) and 90-day survival (76.7 vs 62.3 days, $P = 0.103$) than non-sarcopenic group, although there were no statistically significant differences. GAHS was the most accurate predictive factor for early mortality among DF, ABIC (Age, Bilirubin, INR, Creatinine), Child–Pugh, and Model for end-stage liver disease score (AUROC – 0.785).

Conclusions: Sarcopenia is frequent complication in patients with SAH. Sarcopenia was not associated with early mortality. However, although not significant, sarcopenia appear to be associated with overall survival. In addition, sarcopenia is likely to be associated with HE, which is important prognostic factor for short term mortality.

P1100

SCORING SYSTEMS PROPOSED FOR ACUTE-ON-CHRONIC LIVER FAILURE ACCURATELY PREDICT SHORT-TERM MORTALITY IN PATIENTS WITH ALCOHOLIC HEPATITIS

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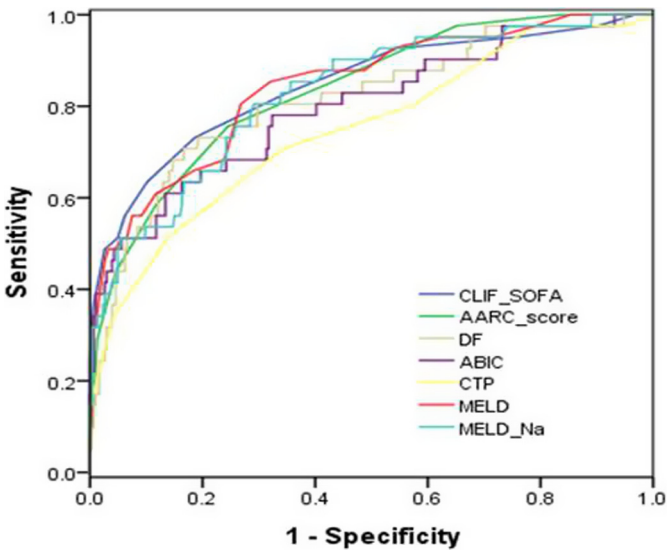
Background and Aims: Alcoholic hepatitis often leads to acute-on-chronic liver failure (ACLF), characterized by acute hepatic decompensation of chronic liver disease, organ failure and high short-term mortality. The aim of this study was to assess the performance of proposed scores specific for ACLF in predicting short-term mortality among patients with alcoholic hepatitisAlcoholic hepatitis often leads to acute-on-chronic liver failure (ACLF), characterized by acute hepatic decompensation of chronic liver disease, organ failure and high short-term mortality. The aim of this study was to assess the performance of proposed scores specific for ACLF in predicting short-term mortality among patients with alcoholic hepatitis

Methods: We retrospectively collected data from 350 patients with acutely decompensated alcoholic liver disease with active alcoholism from January 2013 to December 2013 at 21 academic hospitals in Korea. The performance for predicting short-term mortality was calculated for chronic liver failure-sequential organ failure assessment (CLIF-SOFA), APASL ACLF Research Consortium (AARC) liver failure grade, Maddrey's discriminant function (DF), Age, bilirubin, international normalized ration and Creatinine score (ABIC), Child-Turcotte-Pugh (CTP), Model for end-stage liver failure (MELD), and MELD-Na.

Results: The 28-day and 90-day mortality were 12% and 18%, respectively. The area under receiver operating characteristic curve (AUROC) of CLIF-SOFA, AARC liver failure, DF, ABIC, CTP, MELD, and MELD-Na were 0.85 (0.77–0.91), 0.83 (0.77–0.90), 0.81 (0.74–0.89), 0.80 (0.72–0.88), 0.75 (0.66–0.84), 0.84 (0.77–0.91), and 0.83 (0.76–0.90) for 28-day mortality, and 0.83 (0.77–0.89), 0.81 (0.75–0.88), 0.81 (0.74–0.88), 0.80 (0.74–0.87), 0.74 (0.66–0.81), 0.84 (0.78–0.90), and 0.86 (0.80–0.91) for 90-day mortality, respectively.

Conclusions: The proposed scores specific for ACLF, such as CLIF-SOFA or AARC liver failure score, showed comparable predictive ability for short-term mortality compared to other scoring systems in patients with alcoholic hepatitis



P1101

CYTOCHROME P4502E1 AS A DRIVING FORCE IN THE PROGRESSION OF ALCOHOLIC LIVER DISEASE

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Background and Aims: One mechanism by which alcoholic liver disease (ALD) progresses from pure fatty liver to fibrosis, cirrhosis, and hepatocellular cancer (HCC) is oxidative stress. The induction of cytochrome P4502E1 (CYP2E1) by alcohol results among others in the generation of reactive oxygen species (ROS). Experimental data in rats underline the key role of CYP2E1 since ALD (Gouillon et al, Proc Soc Exp Biol Med 224, 302: 2000) as well as hepatic adenoma development (Ye et al, Hepatobil Surg Nutr 1, 5: 2012) could be partially prevented by the concomitant administration of the specific CYP2E1 inhibitor chlormethiazole. In addition, it was found that CYP2E1 induction leads to the formation of various highly carcinogenic exocyclic etheno DNA-adducts. The purpose of the present study was to investigate CYP2E1 induction in non cirrhotic ALD and to correlate CYP2E1 with various histological parameters including hepatic fat, inflammation, fibrosis, and carcinogenic etheno DNA adducts.
Methods: Hepatic biopsies from 97 patients with a history of chronic alcohol intake (>60 g/day over 20 years) diagnosed as non-cirrhotic ALD were carefully histologically determined using various scores for for steatosis, inflammation, and fibrosis. In addition, CYP2E1, and 1,N^6-etheno-2′-deoxyadenosine (εdA), the most important etheno DNA-adducts, were determined immunohistologically (Wang et al, Hepatology 50, 453: 2009), and correlated with each other.
Results: A significant correlation was found between CYP2E1 and εdA ($p < 0.0003$), between CYP2E1 and fibrosis ($p < 0.03$), and between fibrosis and lobular inflammation ($p < 0.0001$). The amount of alcohol consumed correlated significantly with serum ALT activity, ($p < 0.03$), MCV ($p < 0.02$), inflammation ($p < 0.004$), and fibrosis ($p < 0.05$), but not with CYP2E1.
Conclusions: The data presented emphasize for the first time a causal role of CYP2E1 in the fibrotic progression of ALD and possibly in the pathogenesis of alcohol mediated hepatocarcinogenesis.

P1102

NO HISTOLOGICAL FEATURES OF ALCOHOL INDUCED STEATOHEPATITIS CAN IDENTIFY PATIENTS AT RISK OF CIRRHOSIS AND PREMATURE DEATH

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Background and Aims: Whereas almost all heavy alcohol abusers develop steatosis, only 20–30% progress to cirrhosis. The prognostic impact of early stages of histological ALD on risk of cirrhosis has only sparsely been studied. Our aim was to determine the risk of cirrhosis and premature death in patients with biopsy verified alcoholic steatohepatitis (ASH), and to compare prognosis to alcoholic pure fatty liver (AFLD) and the general population. Secondary aims were to identify clinical, biochemical and histological parameters with prognostic significance.
Methods: Patients with AFLD and ASH diagnosed during the period 1976–1987 were identified through the pathology register at Hvidovre Hospital, Denmark. Medical records were reviewed, and supplementary data was retrieved from the Danish National Hospital Registry and the Registry of Causes of Death. All biopsies were re-examined, morphological findings were assessed regarding steatosis, lobular inflammation, hepatocyte ballooning and fibrosis. Outcomes analysed were overall mortality and a diagnosis of cirrhosis. Cox proportional hazard models adjusted for age and gender were used to analyse the differences in mortality, and the prognostic effect of histological and biochemical parameters.
Results: 225 patients with AFLD and 114 patients with ASH were followed for a median of 13 and 9.7 years. Median age at baseline was 49.8 and 51.2 years, respectively. During follow-up, 93.3% of patients with AFLD died and 55.8% (126/224) developed cirrhosis, whereas 96.5% of patients with ASH died and 58.9% (66/112) developed cirrhosis. Median age at death was 63.7 and 63.6 years, respectively. Mortality was significantly higher in patients with ASH compared to patients with AFLD ($p = 0.03$) and the general population ($p < 0.001$), but no histological or biochemical parameters with prognostic significance for mortality were identified.
Conclusions: Histologically verified alcoholic liver disease is associated with a grave prognosis, and the presence of steatohepatitis indicates an increased mortality compared to patients with pure steatosis. However, none of the histological characteristics defining steatohepatitis can independently identify patients at risk for premature death.

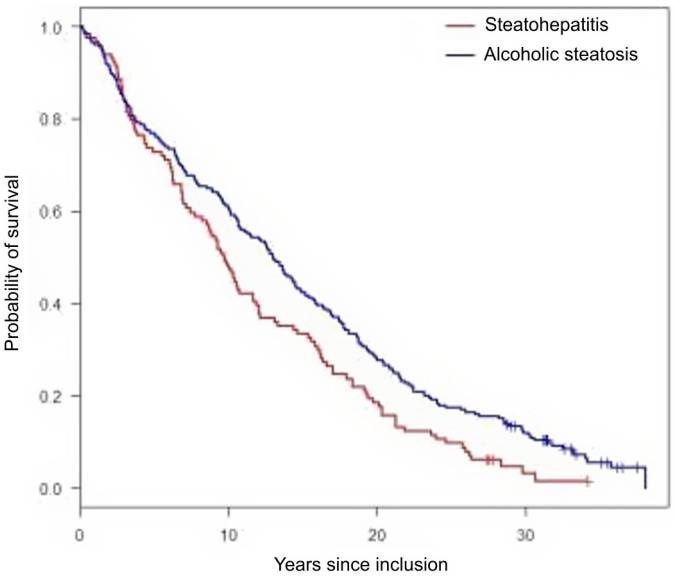

Figure: Kaplan–Meier plot for mortality – alcoholic steatosis and steatohepatitis.

P1103

PORTAL HYPERTENSIVE BLEEDING INDEPENDENTLY PREDICTS MORTALITY IN PATIENTS HOSPITALIZED FOR ALCOHOLIC HEPATITIS

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Background and Aims: Gastrointestinal bleeding (GIB) is often accompanied by patients with alcoholic hepatitis (AH). The aims of the present study were (1) to identify the etiologies of GIB in patients hospitalized for AH; (2) to investigate the clinical characteristics and long-term survival outcomes of patients

hospitalized for AH according to the presence or absence of GIB; (3) to identify factors associated with long-term mortality.
Methods: A hospital-based, retrospective cohort comprised consecutive patients who were hospitalized for AH between 1999 and 2014. Those patients were dichotomized into two groups: those who experienced GIB once or more (GIB group) and those who never experienced GIB till death or censoring (NGIB group). GIB group was dichotomized into another two groups: those whose bleeding focus of the 1st GIB was found to be portal hypertensive bleeding (PHB group) and those of non-portal hypertensive bleeding (NPHB group). Clinical characteristics and long-term survival outcomes of patients hospitalized for AH according to the presence or absence of GIB and PHB were investigated. Risk factors for long-term mortality in AH patients were also analyzed using the Cox regression method.
Results: A total of 331 patients hospitalized for AH were included in this study. Among them, 131 patients experienced GIB at admission or during follow up. Of the 131 patients, the most common cause of GIB was esophageal varix. Using the log rank test, GIB group had worse survival outcome compared with NGIB group (log rank test, p = 0.029) PHB group had worse survival outcome compared with the NPHB group (log rank test, p = 0.001). There was no significant difference in survival rate between the NPHB group and NGIB group. In the multivariable analysis of all AH patients, alcohol dose, ascites, encephalopathy, MDF and MELD were associated with mortality. In the multivariable analysis of GIB group, MELD score (HR, 1.09; 95% CI, 1.055–1.128; $P < 0.001$) and the presence of PHB (HR, 2.37; 95% CI, 1.060–5.277; $P = 0.035$) were found to be independently associated with mortality.
Conclusions: Portal hypertensive bleeding and high MELD scores showed worse survival outcomes in AH patients with gastrointestinal bleeding. Therefore, the prompt endoscopic examination may help physicians to stratify the risk of mortality in AH patients with GIB.

P1104
EXPRESSION OF LIVER-SPECIFIC CYTOCHROME P450 ISOENZYMES AND OXYGENASES IN C3A CELLS PRIOR TO AND AFTER TREATMENT WITH THE ELAD LIVER SUPPORT SYSTEM
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Background and Aims: Acute-on-chronic liver failure (ACLF) and acute liver failure (ALF) are characterized by impaired liver function, multi-organ failure, and high short-term mortality. Cell-based support of liver function could be of therapeutic benefit. The ELAD System provides extracorporeal exposure to human hepatoblastoma-derived C3A cells and is under evaluation in several clinical trials for various liver disease indications. The aims of this study were to characterize the expression levels of liver-specific cytochrome P450 isoenzymes (CYP) and oxygenases in ELAD C3A cells during clinical trial production and after use in clinical treatment.
Methods: Monolayer C3A cells were harvested for mRNA isolation and analyzed on TaqMan Human CYP450 and Oxygenases Array Plates (ABI) using routine RT-PCR methods. ELAD C3A hollow-fiber cartridges were recovered at production maturity and after 5 days of clinical use in alcohol-induced liver disease (AILD) subjects and processed as above. Expression levels vs. controls ($2^{-\Delta\Delta Ct}$ method) were set as ≤0.25-fold decrease and ≥10-fold increase for down- and up-regulation, respectively, using the housekeeping gene GUSB (lowest %CV for all samples).
Results: Approximately two-thirds of the 92 total gene targets evaluated were detected in C3A cells. Among these included CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5, which are collectively responsible for metabolizing nearly 90% of all drugs. Up-regulation in monolayer vs. ELAD cartridge C3A cells was observed for CYP17A1, TYR, CYP7A1, CYP4A11/CYP4A22, CYP2C9, CYP11B2, CYP2A6, CYP2A7, CYP2C19, CYP51A1, and CYP3A7, whereas down-regulation was observed for CYP19A1, CYP24A1, and SC4MOL. Up-regulation in ELAD C3A cells prior to vs. after subject treatment was observed for ALOX5, CYP11B1, CYP11B2, CYP2A6, and CYP2A7, whereas down-regulation was noted for TYR and CYP51A1. C3A cells from subject-recovered ELAD cartridges exhibited differences in up- or down-regulation between individual subjects among 16–22 different gene targets.
Conclusions: This study provides supporting evidence that the C3A cells comprising the ELAD System exhibit a diverse expression of various critical liver metabolic enzymes, and is supportive of previous studies using drug metabolites as evidence of the presence of selected CYPs. It also illustrates that unique responses can be observed in cell-based therapies as a result of dynamic interactions with individual patients' unique physiologies.

P1105
IMMUNOLOGIC BASIS OF CLOPIDOGREL HYPERSENSITIVITY AND DETECTION BY LYMPHOCYTE TOXICITY ASSAY
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Background and Aims: Clopidogrel is a commonly prescribed platelet inhibitor for patients diagnosed with acute coronary syndrome or undergoing stent placement. Hypersensitivity reaction (HSR) after clopidogrel initiation manifesting clinically as elevated transaminases in the presence of dermatotoxicity are recognized complication affecting up to 3% of treated patients.
Our primary objective is to characterize the cellular and immunologic mechanism of clopidogrel HSR and describe a novel lymphocyte toxicity assay (LTA) for the laboratory confirmation of diagnosis in affected individuals. We also aim to understand the immune and pathophysiological mechanism(s) of clopidogrel-HSRs.
Methods: Blood from 19 patients with a clinically confirmed diagnosis of clopidogrel HSR and 6 tolerant controls were analyzed for hematologic and an immunologic response during the active phase of clopidogrel HSR. LTA was performed after a minimum of 12 months post clopidogrel discontinuation. Pro-inflammatory cytokine and anti-inflammatory cytokine profiles and apoptotic (M-30) and necrotic (M65) assays were performed in all patients.
Results: The hematological screen identified a significant increase in circulatory neutrophils and a significant decrease in circulatory lymphocyte counts with no change in the eosinophil counts. The mean percentage LTA values were significantly higher for clopidogrel HSR compared to control patients (20±6 vs. 7±4%, p = 0.0004). The levels of TNF-α levels were also significantly higher for the clopidogrel HSR group compared to controls (116±49 vs. 29±9 pg/mL). ROC curves for LTA demonstrated excellent utility for the diagnosis of clopidogrel HSR (AUC=0.97, p = 0.003) and an LTA >16% showed a sensitivity of 86% and a specificity of 100% for the diagnosis of clopidogrel HSR.
Conclusions: Clopidogrel HSR is a significant immune-mediated phenomenon. This adverse event may be diagnosed and prevented by the use of the laboratory LTA. The LTA to Clopidogrel hypersensitivity provides a framework to study *in vitro* cell assay that is useful for patient diagnosis and drug monitoring.

P1106

ALCOHOL-RELATED LIVER DISEASE PATIENTS' BELIEFS ABOUT THEIR ILLNESS AND FACTORS THAT INFLUENCE THEIR SELF-MANAGEMENT – A PATIENT SURVEY

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Background and Aims: Research in a variety of long term illnesses suggests patients' illness beliefs are a more influential factor for patient recovery than the severity of the illness. However, research into illness belief and medication adherence of patients with alcohol-related liver disease (ALD) is sparse. This study aims to determine the association between illness belief and self-management and, more specifically, adherence to medication and provide the evidence-base for the development of an effective and personalised framework to support self-management in patients with alcohol-related liver disease.
Methods: A cohort of 159 patients with ALD patients who attended the Liver Outpatient Clinic at the King's College Hospital NHS Foundation Trust (October 2012 to November 2013) completed a set of validated instruments measuring illness beliefs, self-management, beliefs about medication, emotional states and quality of life.
Results: The mean age of enrolled patients was 52 years (range 27–80), 67% male, 26% live on their own, 61% had no previous history of other chronic illness. Their average MELD and Audit C scores were 11.0 (range 6–28) and 3.5 (range 0–12) respectively.
Multiple regression analyses were performed on Anxiety (variation explained by Illness perception components adjusting for the demographic and illness characteristic = 42%) and Depression HAD scores (40%), Self-management Chronic illness (47%) and Liver summary scores (25%) and Quality of life ED-5Q your state of health today (25%). The two most consistent illness belief components across all outcome measures were 'comprehension' and 'identity'.
Regression analysis found that the illness perception components were statistically associated with beliefs about medicines questionnaire (BMQ) components: 'comprehension' associated with 'concern' ($p = 0.005$); 'identity' associated with 'necessity' and 'concern' ($p = 0.005$; $p = 0.043$ respectively), and 'treatment control' associated with 'necessity' and 'differential' ($p = 0.047$; $p = 0.039$ respectively).
Conclusions: Interventions designed to improve the understanding of patients with ALD of their illness and strategies to manage their symptoms are likely to improve their self-management, quality of life and reduce anxiety and depression. More specifically, medication adherence in patients with alcoholic liver disease is likely to be improved by an intervention that improves ALD patient's understanding, management of symptoms and sense of control.

P1107

UNCONSIDERED MISUSE OF ACETAMINOPHEN IS ONE OF THE CAUSAL COFACTORS OF ACUTE LIVER FAILURE IN ADULTS WITH SEVERE DENGUE FEVER

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Background and Aims: In recent years, dengue fever extended over various areas beyond tropical and subtropical areas. Fifty to hundred millions cases of dengue occur annually. Acetaminophen (ACT), no more than 4g daily, is officially recommended as an antipyretic. Adults with dengue often have discrete liver blood tests abnormalities but uncommonly develop acute liver failure (ALF). Our aims were to itemize potential cofactors, and determine whether ACT toxicity could be a causal factor, of ALF in patients with dengue.
Methods: Review of previously published and strictly selected case reports of adults with dengue and ALF (DALF), and of the files of similar patients who were refered to us.
Results: Among 61 cases of adults with DALF, the survival rate was 82% or 50%, depending on whether they were, or were not, given intravenous *N*-acetylcysteine (*IV NAC*). Itemized causal factors of DALF include viral infection of liver tissue, virus-related immunopathological mechanisms, acute hypovolemia due to acute plasma leakage, the systemic inflammatory response syndrome and acute episodes of hyperthermia. ACT hepatotxicity was only recently mentioned. Seven adults with dengue, recent ingestion of ACT and serum aminotransferase activity above 25 times the normal value, were hospitalized in 2 liver intensive care units. Four patients with severe coagulopathy but without grade III-IV encephalopathy when they received *IV NAC*, survived; 2 of them had recent unintentional acetaminophen overdose, one developed DALF, the other with centrilobular liver necrosis did not; the 2 other patients, developed mediolobular liver necrosis-associated DALF and one required liver transplantation (LT). Among the 3 patients with grade III-IV encephalopathy and hypotension when they received *IV NAC*, all died after protracted shock; 2 of them had massive liver necrosis, including one rechallenged with ACT despite increased serum aminotransferases after unrecognized ACT unintentional overdose.
Conclusions: in adults with DALF, various morphological kinds of liver necrosis suggest various etiologies, life-saving LT may be required, and unconsidered misuse of ACT may cause ACT-induced hepatotoxicity and DALF. Accordingly, *IV NAC* is beneficial to hospitalized patients with dengue and serum aminotransferases raised 25 times above normal without encephalopathy.

P1108

ELEVATED ACETALDEHYDE LEVELS ARE DETRIMENTAL TO THE HEPATIC ANTIOXIDANT SYSTEM

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Background and Aims: Acute (binge) and chronic alcohol drinking is a major public health issue leading to alcoholic liver disease.

One of the consequences of binge drinking is a marked elevation in acetaldehyde levels, however this effect on the hepatic antioxidant system has not been fully explored and may explain the increased susceptibility to oxidative stress.
Methods: Male Wistar rats were injected with an acute amount of alcohol (75 mmol/kg) pre-treated with and without the acetaldehyde dehydrogenase inhibitor cyanamide (0.5 mmol/kg) and sacrificed after 24 hours. Control animals were injected with saline (0.15 mol/L). Hepatic cytosolic and mitochondrial fractions were prepared and analysed for oxidative stress. Measurements included reduced and oxidised glutathione, oxidised proteins, catalase, glutathione peroxidase, glutathione reductase, superoxide dismutase.
Results: Our findings showed that alcohol alone led to significantly lower glutathione peroxidase and superoxide dismutase activity in the mitochondria and cytosol, with glutathione reductase activity increased in both compartments; catalase activity remained unchanged. This correlated with increased mitochondrial protein oxidation and slightly lower glutathione levels. These effects were exacerbated when acetaldehyde levels were increased, but with catalase activity also significantly lower and corresponded to increased cytosolic protein oxidation and depletion of cytosolic glutathione levels.
Conclusions: In conclusion, some compensatory mechanisms are induced to replenish the lower glutathione levels; however under conditions of elevated acetaldehyde levels, greater alteration to the antioxidant enzyme capacity occurs, leading to significant cytotoxic injury.

P1109
INCIDENCE, MORTALITY, AND READMISSION RATE OF PATIENTS HOSPITALIZED FOR ALCOHOLIC HEPATITIS IN KOREA: A NATIONWIDE EPIDEMIOLOGIC STUDY

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Background and Aims: Alcoholic hepatitis (AH) ranks among the most costly diseases in Korea. However, accurate hospitalization incidence, mortality, and contributing factors have never been investigated yet. This study aims to provide the nationwide incidence of hospitalization, mortality, and readmission rate in patients with AH in Korea.
Methods: Using database of the Korean Health Insurance Review and Assessment service, we identified total inpatient cases based on ICD-10 diagnoses of AH (K70.1) and surveyed concomitant cirrhosis and alcohol use disorder (AUD) from 2008 to 2012. Standardized hospitalization incidence and mortality rates were calculated using data of medical services provided to patients hospitalized for AH. Logistic regression analysis was performed to identify risk factors for in-hospital mortality. Follow-up data for those admitted in 2008 were analyzed to find risk factors for readmission.
Results: The standardized incidence rate of hospitalization per 10^5 population per year decreased from 19 in 2008 to 14 in 2012. The inpatient mortality rate ranged from 0.2 to 0.5% per year. In-hospital mortality was significantly higher in older patients (OR, 1.63; 95% CI, 1.17–1.58) and in those with cirrhosis (OR, 4.4; 95% CI, 2.78–6.94). The readmission rate of patients admitted in 2008 was 34.0%. Male sex (OR, 1.46; 95% CI, 1.24–1.73) and low economic status (OR, 2.34; 95% CI, 2.13–2.57) were associated with readmission, while old age (OR, 0.96; 95% CI, 0.97–0.99), cirrhosis (OR, 0.77; 95% CI, 0.60–0.98), and urban residency (OR, 0.67; 95% CI, 0.59–0.76) were inversely associated with readmission. AUD tended to increase the risk of re-hospitalization but did not reach statistical significance (OR, 1.13; 95% CI, 0.99–1.27; $P = 0.051$).
Conclusions: This study captured the epidemiology of patients hospitalized for alcoholic hepatitis in Korea. These results offer us valuable information to decide on the priorities of public health policies associated with alcoholic liver disease burdens.

P1110
ALCOHOL LIVER DISEASE IS MORE FREQUENT AND MORE SEVERE THAN CHRONIC VIRAL HEPATITIS IN JAILS OF A FRENCH DISTRICT

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Background and Aims: This sub-study was carried out as part of the prospective study PRODEPIST whose objectives were: (1) to promote detection of viral hepatitis, HIV, addictive behaviour, hepatitis B vaccination and (2) to evaluate liver fibrosis.
Methods: HCV and HBV detection were offered to 702 people at entrance and before exit of two prisons from June 2012 to December 2013. Alcohol dependence was assessed with CAGE test (Ramirez et al. 1988). Fibrosis was evaluated by Fibromètre® and/or transient elastometry (Fibroscan®) in patients with chronic hepatitis B or C and/or alcohol dependence since January 2013.
Results: 357 out of 702 detainees (51%) agreed to participate to the PRODEPIST study. 94.1% were male; median age was 30 years (25–39), median duration of detention was 183 days (92–396). A positive HCV serology was found in 4.7% (16/342) of inmates and positive RNA in 1.5% (5/342). Two inmates were from Georgia, 2 from France and 1 from Italy. These 5 patients were treated for 24 weeks by peg-interferon, ribavirin and telaprevir. Two prisoners out of 347 (0.6%) were positive for HBsAg, 7.5% (26/347) had anti-HBc antibodies and 49.3% (171/347) had anti-HBs antibodies. Vaccination was offered to 149 people who had a negative viral serology B; 81 accepted (54.4%). Addictive behaviors were frequent in this population: 83.8% were tobacco smokers (294/351), 42.6% (145/340) smoked cannabis and 24.1% (79/327) had an opiate addiction. 179 out of 337 detainees had consumed alcohol (53%) and 70% of patients evaluated by the CAGE test were alcohol dependent (69/98). Seventy-five prisoners were screened for fibrosis either by Fibromètre® (n = 26) or by Fibroscan® (n = 33) or by the two methods (n = 16). Significant fibrosis was found in 12 inmates (HCV 4 and alcohol 8) out to 75, that is 16% (2 were F2, 3 F3 and 7 F4). In the group of prisoners with alcohol dependence, 12.5% (8/64) had significant fibrosis (≥F2).

	Assessment of liver fibrosis (total number=75; nb alcohol=64; nb HCV=10; nb HBV=1)				
Evaluation method	Fibromètre® N=26	Fibroscan® N=33	Fibroscan® and Fibromètre® N=16	Total N=75	%
< F2	20	30	13	63	84.0
Alcohol	18	29	10		
HCV	2	1	2		
HBV	0	0	1		
F2	1	0	1	2	2.7
Alcohol	1	0	1		
HCV	0	0	0		
F3	1	2	0	3	4.0
Alcohol	0	2	0		
HCV	1	0	0		
F4	4	1	2	7	9.3
Alcohol	3	1	0		
HCV	1	0	2		

16%

Conclusions: HCV prevalence was 4.7% (comparable to the study PREVACAR 2010, 4.8%). Prevalence of HBS Ag is very low, and vaccination has been performed in 50% of inmates with negative HBV serology. Alcohol is a major problem in this population (53% were alcohol consumers and 70% are dependent) and is the leading cause of fibrosis in prison (12% of these abusers have significant fibrosis).

P1111

VARIABLES ASSOCIATED WITH ALCOHOL RELAPSE AND PSYCHOSOCIAL INTERVENTIONS TO PREVENT ALCOHOL RELAPSE IN LIVER TRANSPLANT PATIENTS FOR ALCOHOLIC LIVER DISEASE: A SYSTEMATIC REVIEW

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Background and Aims: Liver transplantation is the primary treatment choice for alcohol liver disease. However, alcohol relapse is a particular problem for this population with a reported 10–50% risk. This review aims to identify alcohol relapse variables, and establish the effectiveness and explore the active ingredients of psychosocial interventions in preventing alcohol relapse, in alcohol liver disease patients pre or post-liver transplant.
Methods: Mixed method systematic review involving three parallel syntheses: (1) alcohol relapse variables; (2) psychosocial intervention effectiveness; (3) active ingredients of these psychosocial interventions (i.e. a component analysis). Medline, CINAHL, EMBASE, and PsychInfo were searched in November 2013 for published literature. Grey literature searched in Web of Science, Clinical Trials Register, and Electronic Theses Online Service.
Synthesis 1: Systematic search for and appraisal of prospective studies, retrospective studies, and cross-sectional surveys. Syntheses 2/3: Systematic search for and appraisal of 'randomised controlled trials', 'controlled before and after studies', and 'before and after studies in a single group'.
Results: 23 papers included: 10 cohort, 11 case–control, 1 qualitative, and 1 randomised controlled trial.
Five variables out of nineteen were alcohol relapse predictors (i.e. Synthesis 1): (1) <12 months pre-transplant abstinence; (2) presence of children; (3) poor pre-transplant psychosomatic evaluation; (4) non-compliant with post-transplant treatment plan; (5) active insurance policies at transplant.
One psychosocial intervention paper did not report treatment effectiveness (i.e. Synthesis 2). The remaining three papers reported relapse rate reduction: Alcohol Addiction Unit (odds ratio 0.23), Structured Management (odds ratio 0.32), pre- and post-transplant Substance Abuse Treatment (odds ratio 0.27 compared to no substance abuse treatment; odds ratio 0.23 compared to pre-transplant substance abuse treatment only). With confidence intervals not reported, the uncertainty level around the odds ratio is unclear. Furthermore, a theoretical basis was not discussed; thus, the active ingredients could not be identified (i.e. Synthesis 3).
Conclusions: Randomised controlled trials to further investigate the predictive validity of the five main variables and ascertain the long-term benefits of the tentative yet promising results of the most effective intervention i.e. structured management.

P1112

BACLOFEN: MAINTENANCE OF ABSTINENCE IN ALCOHOL DEPENDENT PATIENTS ATTENDING LIVER CLINIC

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Background and Aims: Alcohol induced liver disease (ALD) is the predominant cause of alcohol-related mortality in the UK. Therefore helping patients with ALD to quit is a primary treatment goal. However, current licensed pharmacotherapies are contraindicated for patients with ALD. Baclofen has shown efficacy in the promotion of abstinence in patients with severe alcohol dependence including those with ALD.
Aim: The primary aim of this study was to measure the effectiveness and tolerability of Baclofen in maintaining abstinence in this difficult to treat group, and to determine if this resulted in a reduction in standard measures of liver damage gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), bilirubin (BiL) and fibroelastography (fibro).
Methods: An observational prospective clinical audit was performed. Patients with ALD were transferred to our hepatology alcohol clinic (HAC) for ongoing support and treatment, and commenced on Baclofen at 10 mg three times daily (TDS), titrated according to tolerability and response up to 30 mg TDS. Primary outcome measures were severity of physical dependence (SADQ score) and biochemical markers of liver damage. These were compared at baseline, and 1 year.
Setting: Acute Hospital Trust.
Participants: 243 patients referred to Hepatology for investigation of abnormal liver function and heavy drinking.
Results: Of the 243 patients commenced on Baclofen in our HAC, 151 (85 female, 66 male) have completed 1 year follow-up (F/U) of which 130 (86%) have remained engaged. 10 have died. All patients had a diagnosis of ALD of which 67 had cirrhosis, 15 had Hep C and 9 had pancreatitis. Comparison of baseline (B/L) and 1 year biochemical markers showed a reduction in GGT ($\chi^2 = 66.8$, $P < 0.0001$) and BiL ($\chi^2 = 82.6$, $P < 0.0001$). There was also in significant difference in mean ALT ($P = 0.005$, 95% CI = 3 to 16). Fibro scores between B/L and F/U were available for 22 patients with 19 scans showing improvements ($\chi^2 = 20.4$, $P < 0.0001$). Between B/L and F/U there was a significant reduction in alcohol consumption ($P < 0.0001$, 95% CI = 10 to 22). And the presence of physical dependence ($\chi^2 = 77.4$, $P < 0.0001$) as categorised by SADQ.
Conclusions: Baclofen is well tolerated in this very difficult to treat, high risk patient group. It has a positive impact on alcohol consumption, and overall measures of liver function and harm. A RCT is needed to confirm the benefit of Baclofen in this patient group.

P1113

HUMAN KERATIN 8 VARIANTS PROMOTE MOUSE ACETAMINOPHEN HEPATOTOXICITY COUPLED WITH JNK ACTIVATION AND PROTEIN ADDUCT FORMATION

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Background and Aims: Keratins 8 and 18 (K8/K18) are the intermediate filaments proteins of simple-type digestive epithelia,

and provide important cytoprotective function. K8/K18 variants predispose humans to chronic liver disease progression and to poor outcomes in acute acetaminophen (APAP)-related liver failure. Given that K8 G62C and R341H/R341C are common K8 variants in European and North American populations, we studied their biological significance using transgenic mice.
Methods: Mice that overexpress the human K8 variants R341H or R341C were generated and used together with previously described mice that overexpress wild-type (WT) K8 or K8 G62C. Mice were injected with 600 mg/kg APAP intraperitoneally, or underwent bile duct ligation (BDL). Livers were evaluated by microarray analysis, quantitative RT-PCR, immunoblotting, histological and immunological staining, and biochemical assays.
Results: Under basal conditions, the K8 G62C/R341H/R341C variant-expressing mice did not show an obvious liver phenotype or altered keratin filament distribution, while K8 G62C/R341C animals had aberrant disulphide-crosslinked keratins. Animals carrying the K8 variants displayed limited gene expression changes but had lower nicotinamide N-methyl transferase levels and were predisposed to APAP-induced hepatotoxicity. The more pronounced liver damage was accompanied by increased and prolonged JNK activation; elevated APAP protein adducts; K8 hyperphosphorylation at S74/S432 with enhanced K8 solubility; and prominent pericentral keratin network disruption. No differences in APAP serum levels, liver inflammation, glutathione or ATP levels were noted. BDL resulted in similar liver injury and biliary fibrosis in all mouse genotypes.
Conclusions: Expression of the human K8 variants G62C, R341H, or R341/C in mice predisposes to acute acetaminophen hepatotoxicity, thereby providing direct evidence for the importance of these variants in human acute liver failure.

P1114

A METABOLOMICS APPROACH TO IDENTIFY EXCRETORY METABOLITE SIGNATURE IN PATIENTS WITH SEVERE ALCOHOLIC HEPATITIS NON RESPONSIVE TO GLUCO-CORTICOSTEROID THERAPY

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Background and Aims: Severe alcoholic hepatitis (SAH) still remains a major cause of liver related mortality. In the absence of a suitable therapy, beneficial role of corticosteroid therapy has been well documented, though some patients do not respond to treatment (recognizable only after 7 days post-treatment). The aim of this study was to identify the urinary metabolite signature capable of predicting non-responsiveness to the therapy at baseline and at follow-up. We also intended to gain insights into molecular mechanisms that are associated with non-response to corticosteroids.
Methods: A comprehensive urinary metabolome profiling of SAH patients (n = 60) was performed by high resolution UPLC-MS at baseline, Day 4 and Day 7. Urine samples were classified into discovery phase [responders (R; n = 20) and non-responders (NR; n = 3)] and the results validated on 37 blinded samples (R, n = 32; NR, n = 5). Multivariate analysis was used to identify significantly different metabolites between R and NR, followed by correlation of metabolic signatures with pathways involved and clinical outcome.
Results: A total of 1250 metabolites were annotated [KEGG-HMDB-METLIN databases]. Of them 183 metabolites were validated based on spectral analysis on MRM. In positive mode, 16%, 20% and 26%, while in the negative mode 10%, 30% and 36% metabolites varied significantly ($p < 0.05$) at day-0, day-3 and day-7 respectively between R and NR. A set of metabolites could categorically and significantly identify non-responders at baseline. Some of the key metabolites belonged to primary and secondary bile acids, tryptophan-metabolic pathway and arginine-proline-metabolic pathway. Enrichment analysis identified metabolites to be of hepatocytic origin [$p < 0.01$], and were predicted to be associated to fumaric aciduria, bilary aterisa and cirrhosis [$p < 0.05$]. Validation of these metabolites on blinded samples confirmed the findings. PLS-DA and cluster analysis differentiated NR from R significantly. Levels of the metabolic signature significantly correlated to the severity indices and Lille score [$r^2 > 0.3$, $p < 0.05$] respectively.
Conclusions: The metabolic composition of SAH patients varies with time and treatment with steroids. The metabolites were able to stratify NR from R even at baseline. The proposed metabolomic approach may become a clinical tool for early assessment of treatment response in severe alcoholic hepatitis.

P1115

ERK AND P38 ACTIVATION ARE CENTRAL MEDIATORS OF IRINOTECAN-INDUCED STEATOHEPATITIS

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Background and Aims: Preoperative chemotherapy for hepatic resection of colorectal liver metastases is associated with the development of chemotherapy-associated steatohepatitis (CASH) which increases the risk of perioperative morbidity and mortality. Here we aimed to unravel the molecular mechanisms of irinotecan induced CASH.
Methods: Primary human hepatocytes were treated with irinotecan *in vitro*. Mice were injected with irinotecan (50 mg/kg every three 3 days for 3 weeks), and control mice were injected with vehicle only. Furthermore, we analyzed liver tissues of previously untreated patients with metastatic colorectal cancer and without underlying liver disease (i) treated with single-agent irinotecan (n = 6) or (ii) without chemotherapy (n = 6).
Results: Irinotecan caused a dose-dependent induction of lipid accumulation and pro-inflammatory gene expression *in vitro*. Furthermore, irinotecan induced the accumulation of free fatty acids, the formation of reactive oxygen species (ROS) and ERK-activation. Pre-incubation with ROS-scavengers or ERK-inhibitor almost completely inhibited irinotecan-induced inflammatory gene expression but had only a slight effect on lipid accumulation. Analysis of autophagy related genes, LC3 modification and p62 degradation revealed that irinotecan dose dependently inhibited autophagy, a cellular mechanism known to affect cellular lipid homeostasis. MAPK p38 is a known inhibitor of autophagy and we found that irinotecan induced p38 activation while pretreatment with a p38 inhibitor diminished irinotecan effects on autophagy as well as on cellular lipid accumulation in hepatocytes *in vitro*. Also in the liver of mice and patients treated with irinotecan we found significant steatosis, ROS-formation and inflammation accompanied by increased ERK- and p38-activation and a concomitant reduction of markers of autophagy in comparison to normal liver tissue.
Conclusions: ERK and p38 activation are critical mediators of irinotecan-induced steatohepatitis which suggests inhibition of these pathways as promising therapeutic regime for prevention of CASH.

P1116
ARE MEGAMITOCHONDRIA A CELLULAR SURVIVAL STRATEGY FOR ETHANOL-INDUCED LIVER TOXICITY?

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Background and Aims: A characteristic histological feature of alcoholic liver disease (ALD), known since the 1970s, is the presence of megamitochondria (MM) in hepatocytes. In a recent study conducted in more than 100 patients, the presence of MM was associated with a lower risk of death within 90 days (Altamirano *et al.*, Gastroenterology, 2014). Mitochondria are dynamic organelles and their shape is intimately involved with their function, under physiological conditions the changes in mitochondrial morphology are regulated by mitochondrial-shaping proteins (MSP). However, the mechanisms driving MM formation, their role, function and involvement in the pathogenesis of ALD is currently unknown, which is the aim of this study.
Methods: Human precision cut liver slices (PCLS) and hepatoma cells VL-17A (positive for ADH/CYP2E1) were cultured with EtOH alone or in combination with FFA, to induce hepatotoxicity. Cell viability was evaluated by FACS (AnnexinV/PI positive cells) or ATP quantification. Post-treatment, changes in the mitochondrial shape were assessed by confocal and electron microscopy; MSP expression and activation was also analysed by Western blot on isolated mitochondria. Additionally, mitochondrial functionality was evaluated in intact cells using the Seahorse Bioscience analyser. Changes in inflammatory cytokine production were assessed by Cytokine Bead Array.
Results: The toxic effect of EtOH (+/− FFA) was confirmed by an increase in the percentage of dead cells or a reduction in ATP levels. Cells that survived the toxic insult showed a dramatically increased proportion of MM; however they also had a compromised ability to grow. Mitochondrial functionality, in terms of coupling efficiency and spare respiratory capacity, was also impaired. Pro inflammatory cytokine profile was found in the supernatants of these cultures. This was associated with a reduced expression and activity in the MSP related to mitochondrial fragmentation.
Conclusions: Cellular survival strategies, in the face of a chronic toxic stimulus, are a necessary requirement to ensure continuing functionality of the organ. We show here that growth retarded hepatocytes, which survived the EtOH insult, are characterized by a dramatic formation of MM, suggesting this as a mechanism to avoid death. We also show for the first time the involvement of MSP in this pathway, raising the potentiality for the use of these proteins as therapeutic targets and biomarkers in ALD.

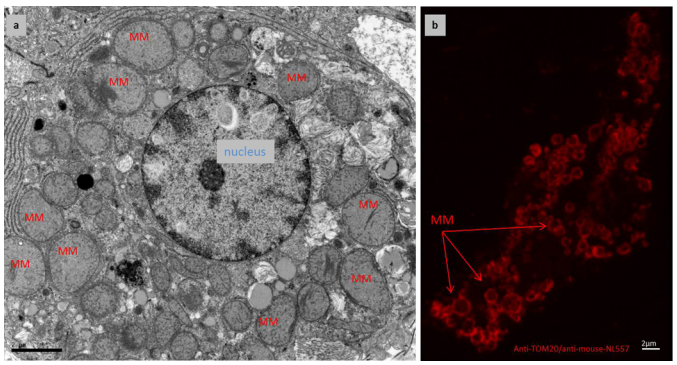

Figure: Ethanol-induced megamitochondria (MM) formation visualized (a) by electron microscopy in human PCLS and (b) by confocal microscopy in VL-17A cells.

P1117
HISTOLOGIC FINDINGS IN LIVER BIOPSY OF ALCOHOLIC CIRRHOSIS PATIENTS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background and Aims: So far there is no effective antifibrotic treatment in alcoholic liver cirrhosis (ALC). One of the novel treatment options is using of autologous hematopoietic stem cells (HSC). Sinusoids capilliarization and myofibroblasts activation are key events in liver fibrogenesis. The purpose of the study was evaluation of the effect of HSC autotransplantation on these processes in patients with alcoholic cirrhosis.
Methods: The study was performed on liver biopsies of 12 patients with ALC taken before the injection of autologous peripheral blood HSCs mobilized by GCSF into celiac trunk, 3 and 12 months after the procedure. Biopsies were stained immunohistochemically with antibodies against CD34 and a-SMA. CD34 is absent in endothelial cells of normal liver however it appears in case of their capillarization. A-SMA is a marker of myofibroblasts.
Results: Before transplantation we observed the great number of CD34-positive cells predominantly in portal areas as well as some positive cells in septa and in parenchyma. A-SMA-positive myofibroblasts were mainly localized in periportal zones and portal tract infiltration areas. 3 months after transplantation the number of CD34-positive cells and myofibroblasts markedly decreased; remaining cells were located mainly around portal tracts. 12 months after transplantation the number of CD34 and a-SMA-positive cells increased again but didn't reach initial levels. The number of cells correlated with the severity of infiltration.
Conclusions: We suggest that transplantation of HSCs in patients with ALC is safe and effective procedure leading to decreasing of myofibroblasts number and restoring of normal sinusoids' structure. However this procedure should be probably repeated in a year.

P1118
Hsp72 OVEREXPRESSION PROTECTS FROM ACETAMINOPHEN AND MCD LIVER INJURY

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Background and Aims: Heat shock protein (Hsp) 72 is a molecular chaperone which is upregulated in a response to a variety of stress situations and possesses broad cytoprotective functions. The hepatic function of Hsp72 remains largely unknown.
Methods: To study the importance of Hsp72 in the liver, we generated transgenic mice overexpressing Hsp72 under the control of a tissue-specific tetracycline-inducible system and crossed them with animals carrying the tetracycline-responsive transactivator under the control of the liver activator protein promoter (Hsp72-LAP mice). Acute liver injury was induced by a single intraperitoneal injection of acetaminophen (800 mg/kg). Long-term feeding (8 weeks) with methionine choline-deficient diet (MCD) was used to induce lipotoxic liver damage.
Results: Hsp72-LAP mice displayed doxycycline-regulated, robust Hsp72 overexpression in hepatocytes, but not in the other tissues or cell types. Eighteen hours after acetaminophen injection, a significantly lower liver injury was noted in Hsp72-LAP mice in comparison to single transgenes (ALT: 933 vs. 1977, $p < 0.05$). No differences in acetaminophen metabolism were seen, but overexpression of Hsp72 protected from formation of APAP protein adducts ($p = 0.027$) and JNK hyperphosphorylation. After MCD-

feeding, Hsp72-LAP mice displayed lower ALT levels than wild type animals (72 vs. 314, p = 0.02). Overexpression of Hsp72 did not affect the extent of MCD-diet induced metabolic changes but ameliorated MCD-induced NF-κB activation and the resulting complex and hepatic inflammation.
Conclusions: Our results suggest that Hsp72 overexpression protects against specific modes of liver injury.

P1119
ELEVATED LEVELS OF CIRCULATING BACTERIAL DNA IN ALCOHOLIC HEPATITIS IDENTIFIES PATIENTS WHO WILL NOT RESPOND TO CORTICOSTEROID

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Background and Aims: Prednisolone has been shown to improve 28-day survival in alcoholic hepatitis (AH). However, use of this therapy is associated with increased infections that could explain the limited benefit of corticosteroid beyond 28 days. Interestingly, a large body of data suggests that bacterial translocation plays an important role in the pathogenesis of AH. The present study sought to elucidate the impact of prednisolone in patients with elevated circulating bacterial DNA (bDNA).
Methods: DNA was extracted, pre-treatment (Day 0), from the whole blood of 156 patients recruited to the multi-centre *Steroids or Pentoxyfilline for Alcoholic Hepatitis* (STOPAH) study. Patients were matched for MELD, age, gender, therapy and the presence of infection. Subsequently, real-time PCR was performed using a Taqman probe targeting a region within a 380bp fragment of bacterial 16S rDNA for bDNA quantification. Levels of bacterial DNA were compared to Maddrey's discriminant function (mDF), SIRS score, Lille score, the development of an infectious serious adverse event within the first 7 days of therapy, and mortality.
Results: bDNA levels correlated with mDF although this did not achieve statistical significance [r = 0.146, $p = 0.07$]. However, there was strong correlation between bDNA levels and Lille model [r = 0.29, $p = 0.0016$]. The Day 0 level of circulating bDNA in Lille non-responders (>0.45) was higher than in Lille responders (<0.45) [21 vs 14 pg/ml; $p = 0.02$]. Importantly, this association was only seen in patients treated with prednisolone [prednisolone treated: r = 0.4, $p = 0.0046$ vs no prednisolone: r = 0.18, p = ns]. Day 0 bDNA level >18.4 pg/ml in prednisolone treated patients was strongly associated with Lille non-response [odds ratio 6, $p < 0.0001$].

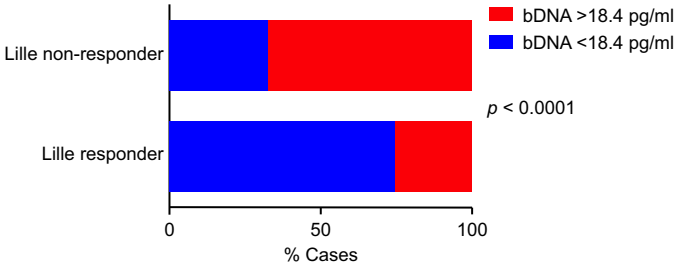

Figure: Pre-treatment bacterial DNA level predicts subsequent Lille response in alcoholic hepatitis.

There was no association between Day 0 bDNA level and the development of infection (22 vs 19 pg/ml, p = ns); SIRS criteria (r = 0.02, p = ns), 28-day or 90-day mortality (18 vs 21 pg/ml, p = ns and 17 vs 21 pg/ml, p = ns).
Conclusions: Pre-treatment circulating bDNA levels may be used to predict poor outcome in AH. Further, patients with elevated circulating bDNA are unlikely to respond to prednisolone therapy and so could be spared the infectious complications associated with this agent. Further work should validate these findings in a larger cohort of AH patients.

P1120
DAY-4 LILLE SCORE – FOR EARLY PREDICTION OF STEROID RESPONSE IN SEVERE ALCOHOLIC HEPATITIS

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Background and Aims: Steroids are the mainstay of treatment of severe alcoholic hepatitis (SAH). Introduction of the Lille score has helped in identification of steroid non-responders (Lille score >0.45) as on day 7. This however requires prolonged hospitalization and often increases the risk of infection. This study was conducted to assess the correlation of day-4 Lille score with day-7 Lille score, so as to define response to steroids early.
Methods: All consecutive patients with SAH admitted at ILBS between March 2013 and March 2014, without contraindications to steroids were enrolled into a prospective study to receive 40 mg prednisolone per day. The steroid response was assessed at day 7 by Lille score as responder (<0.45) or non-responder (>0.45). Lille score was also calculated on day 4 using bilirubin level as per day 4 in the place of day 7 bilirubin value, using the online Lille model formula (www.lillemodel.com).
Results: Of 164 patients with SAH during the study period, 50 patients were started on steroids – men (98%), mean age 39.4±8.9 yr. At admission, ascites was present in 48 (96%), bilirubin 19.3±10.3 mg/dl, creatinine 0.53±0.25 mg/dl, albumin 2.43±0.43 g/dl and PT 24.38±4.99 s. Alcoholic hepatitis severity parameters were DF 77.8±26.6, MELD 24.9±3.67, MELDNa 28.6±4.46, GAH 8.14±0.8, and CTP 10.96±0.92. 40 out of 50 responded to steroids (80%) with a Lille score of <0.45.

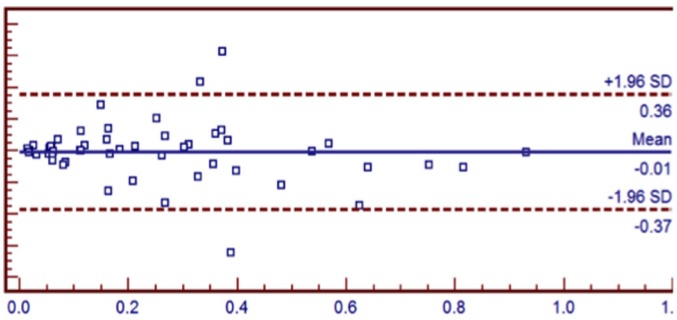

Figure 1. Average of Lille score-day 4 and Lille score-day 7*. *The Bland–Altman graph (only 3 outliers).

Mean Lille score on day 7 was 0.255±0.22 and on day 4 was 0.261±0.25. The paired difference in the day 4 and day 7 Lille score was 0.0065±0.186, p = 0.80, meaning thereby that both the scores are comparable. The day 7 Lille score could be predicted from the day 4 Lille score by using the formula, Day 7 Lille = 0.923 × day 4 Lille. The coefficient of correlation (R) between day-4 and day-7 Lille score was 0.864 with R^2 as 0.747. The reliability coefficient of day 4 Lille score in predicting the day 7 Lille score was 0.825 (CI 0.695–0.902).

The agreement between day 4 and day 7 Lille scores was 0.827 (95% CI 0.695–0.902, p ≤ 0.001). Bland and Altman graph showed agreement between Lille score day 4 and day 7 in 47 patients (94%).
Conclusions: Day 4 Lille score is equally good in predicting the steroid response and hence can be used in place of the currently used day 7 score, so that steroid response could be assessed early for deciding future treatment options for non-responders, such as GCSF or liver transplantation.

P1121

HOMOZYGOSITY FOR THE Ile148Met VARIANT IN PNPLA3 IS SIGNIFICANTLY ASSOCIATED WITH REDUCED SURVIVAL FOLLOWING AN EPISODE OF SEVERE ALCOHOLIC HEPATITIS

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Background and Aims: The non-synonymous variant Ile148Met (I148M, rs738409) in PNPLA3 is associated with an increased risk of developing significant alcohol-related liver injury. Recently it has been shown that carriage of this variant confers a significant negative effect on survival in patients with established alcohol-related cirrhosis (Way et al. Hepatology 2014; 60(Suppl 1): 781A–782A: 1203abs). Alcoholic hepatitis is a distinct clinical syndrome characterised by jaundice and liver impairment often arising on a background of established cirrhosis. In those with severe disease, defined by a Maddrey discriminant function ≥32, mortality may exceed 30% at 28 days. Abstinence from alcohol is a major determinant of long-term outcome in those who survive initially. It is not known whether carriage of the I148M mutation in PNPLA3 further influences outcome.
Methods: The rs738409 PNPLA3 variant was genotyped in 899 British cases with severe alcoholic hepatitis, recruited through the *Steroids or Pentoxifylline for Alcoholic Hepatitis* – STOPAH trial and in 1,188 white British and Irish controls with alcohol dependence but no evidence of liver injury, despite prolonged drinking, recruited at UCL. Patients who survived at least 90 days after initial presentation and in whom 1-year survival data were available (n = 554) were classified into four groups based on their PNPLA3 IL148Met genotype (minor allele G) and their subsequent drinking behaviour categorized as abstinent or drinking. Kaplan–Meier analysis was used to examine the relationship between to PNPLA3 Ile148Met genotype and survival; patients were censored at death/transplantation; the log rank test was used to assess differences in survival curves.
Results: A significant increase in the mean allele frequency of rs7254880 was observed between cases and controls (29.4% vs 18.8%, $p = 5.74 \times 10^{-15}$; odds ratio 1.80). Overall 1-year mortality in the group surviving beyond 90 days was 19.9%. Individuals homozygous (GG) for the 148Met allele in rs738409 had a significantly decreased 1-year survival despite abstinence from alcohol compared with their counterpart (CC vs. GG, 10.3% vs. 28.6%, p = 0.035; CG vs. GG, 11.9% vs. 28.6%, p = 0.046).
Conclusions: In patients surviving an episode of alcoholic hepatitis, homozygosity for the mutant allele in rs738409 is associated with a significantly increased risk of mortality at 1 year despite abstinence from alcohol. Genotyping may help identify individuals more at risk in whom additional support may be required.

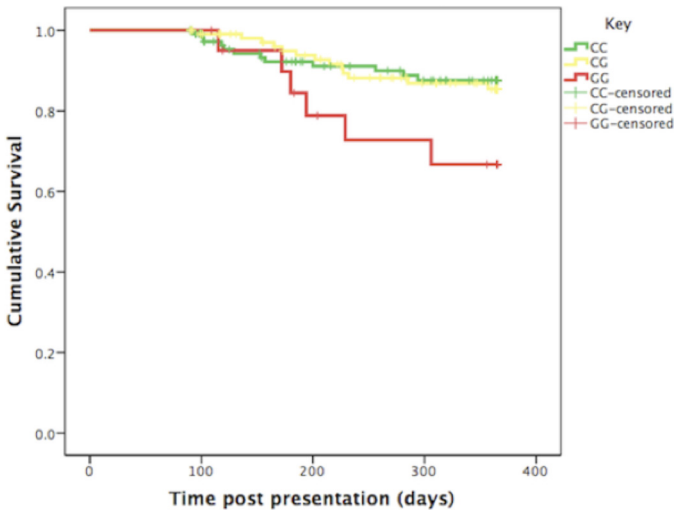

Figure: Kaplan–Meier survival curves. Abstinent drinkers following severe alcoholic hepatitis grouped by rs738409 genotype.

P1122

ALTERNATIVELY ACTIVATED M2 MACROPHAGES PROMOTE HEPATOCYTE DIFFERENTIATION IN HEPATIC PROGENITOR CELL MEDIATED LIVER REGENERATION IN ACUTE ON CHRONIC LIVER FAILURE PATIENTS

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Background and Aims: Macrophages play myriad roles in liver degeneration and regeneration. The changing microenvironment of different setting of liver disease affects the polarization of macrophage and how different subtypes affects hepatic regeneration remains unclear. Aim: To understand the effect of liver microenvironment on the plasticity of liver macrophage and their role in regeneration.
Methods: M1/M2 macrophage marker genes expression by using qRT-PCR in biopsy/explants in ACLF, ALF, CLD. Ki67, CK-19 staining for the nature of liver regeneration. CD68 (tissue macrophages) CD163 (M2 macrophages) for analysis of macrophages in liver tissue from patients with ACLF, ALF, and CLD (n = 15, 21 and 22). Expression of CD68, CD163 were correlated with hepatocyte self-replication, HPC activation and maturation.
Results: qRT-PCR result show increase in M2 gene markers CD163, CD206 & TGM2 and decrease in M1 markers iNOS & CD80 in ACLF comparison to CLD, and increase in M2 gene markers CD163, CD206 & TGM2 and decrease in M1 markers in iNOS & CD80 in ACLF comparison to ALF. IHC analysis shows increase in Ki67+ hepatocytes in ALF vs ACLF. Further, the number of CK19+ HPC and its maturational lineages was increased in ACLF than ALF and CLD. Spearman correlation showed CD163 positivity and M2/M1 ratio is associated with HPC differentiation to hepatocyte. PCR analysis of PU.1 and Myb suggest that increase in PU.1 expression in ACLF in comparison to ALF & CLD suggesting that major population of M2 in ACLF are kupffer cells.
Conclusions: M2 are the major population in ACLF which are kupffer cell origin and promotes differentiation of HPC to hepatocyte.

P1123

A FUNCTIONAL VARIANT IN TM6SF2 ASSOCIATES WITH ALCOHOL-RELATED CIRRHOSIS RISK IN A BRITISH AND IRISH POPULATION

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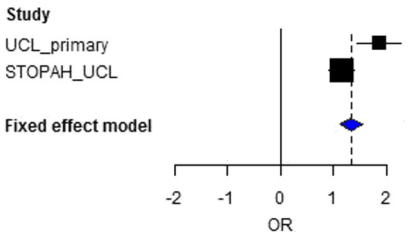
Background and Aims: A large region of linkage disequilibrium at 19p13, containing several genes including *NCAN* and *TM6SF2,* has been significantly associated with multiple lipid-related traits as well as non-alcohol-related fatty liver disease (NAFLD) and alcohol-related liver disease (ALD). A recent exome wide association analysis of NAFLD, identified the SNP rs58542926 (Gln167Lys) in *TM6SF2* as the primary variant driving associations within the 19p13 region. It is known that both NAFLD and ALD share a common genetic background but it is not known whether rs58542926 in *TM6SF2* influences ALD risk.
Methods: The exploratory population (UCL exploratory) comprised 317 cases with biopsy-proven alcohol-related cirrhosis and 330 controls who had been misusing alcohol for >15 years but had no evidence, on biopsy, of alcohol-related liver injury; all were of British or Irish extraction. The replication population (STOPAH-UCL replication) comprised 819 cases with clinically diagnosed severe alcoholic hepatitis, recruited through the *Steroids or Pentoxifylline for Alcoholic Hepatitis* (STOPAH) trial and 847 controls with an extensive history of alcohol misuse but no significant liver damage; all were of British or Irish descent. The SNP rs58542926 in *TM6SF2* (minor allele T) was genotyped using K-Biosciences Competitive Allele Specific PCR genotyping platform. Primary allelic association and fixed-effects meta-analyses were performed in PLINK v1.7. Forest plots were generated in the R package "meta".
Results: A highly significant allelic association was observed in the UCL exploratory population samples between the mutant allele in rs58542926 and cirrhosis risk (minor allele frequency cases 10.7%: controls 6.1% [$p = 0.0026$, odds ratio = 1.86]). This allelic association was not significant in the replication cohort although the directionality of effect mirrored that in the exploratory population ($P = 0.27$, odds ratio = 1.15). A fixed effects meta-analysis showed thatrs58542926 remains significantly associated with alcohol-related liver disease risk ($P = 0.012$, odds ratio = 1.31; Figure).
Conclusions: These analyses provide the first evidence that the functional SNP rs58542926 in *TM6SF2* significantly associates with alcohol-related liver disease risk.



P1124

PRIMARY HEPATOCELLULAR DAMAGE LOWERS ALCOHOL CONSUMPTION AND SUPPRESSES PERIPHERAL FAT MOBILIZATION IN ALD PATIENTS WITH GENETIC VARIANT I148M IN PNPLA3

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Background and Aims: PNPLA3 GG has been identified as major genetic progression factor of ALD and NAFLD, however, its function remains largely unknown. To get more insights into the (patho)physiology of PNPLA3, we here studied 180 clinical, histological and molecular parameters in a large well-characterized ALD cohort prior and after alcohol withdrawal.
Methods: 512 patients with ALD and a mean daily alcohol consumption of 181 g/day were genotyped for PNPLA3. In addition, laboratory tests, liver stiffness (LS) and steatosis (CAP) were measured prior and after 5–6 days of complete alcohol withdrawal. Liver histology was obtained in 80 patients with additional immunostainings for LD-associated proteins in 47 samples. Expression of mRNA transcripts was analyzed in 24 patients.
Results: In contrast to PNPLA3 CC (39.2%) and GC carriers (52.6%), GG carriers (8.2%) drank less alcohol over a shorter time and almost no liquor ($P < 0.001$) without showing a pronounced NAFLD phenotype (weight, BMI, diabetes). LS was significantly elevated in G carriers (median 18 vs. 11 kPa) and highly correlated with histological signs of fibrosis ($r = 0.800$) and liver damage (ballooning, $r = 0.700$; lobular inflammation, $r = 0.340$) but not with steatosis. In CG carrier, LS decreased significantly from 17.6 to 12.7 but to a lesser extent in GG carriers most likely due to a slower resolution of liver damage. Moreover, hepatic fat content (CAP) was only slightly higher in GG carriers and decreased equally in all genotypes by 30 dB/m. Surprisingly, key molecules that are important for lipolysis and backflow of free fatty acids to the liver were drastically reduced in the G carriers. This included the liver-synthesized serum protein ApoA1 and both hepatic mRNA and protein levels of the lipid droplet-associated protein Plin5 and the recently identified hepato-protective factor transcriptional cofactor transducin beta-like-related 1 (TBLR1).
Conclusions: In patients with ALD, PNPLA3 GG status seems to primarily cause hepatocyte damage rather than steatosis per se resulting in a reversible, inflammation-mediated increase of LS. Thus, PNPLA3 GG-associated liver damage could be an important novel factor contributing to impaired peripheral fat mobilization in humans.

P1125

ALCOHOL INDUCES PATHOLOGICAL AS WELL AS PROTECTIVE MECHANISMS IN STEATOTIC HEPATOCYTES

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Background and Aims: Epidemiologic studies proposed an effect of even moderate alcohol consumption on the risk to develop non-alcoholic steatohepatitis (NASH) as well as on the progression from simple steatosis to NASH in obese individuals.

The aim of this study was to establish an *in vitro* model to study the effect of moderate alcohol levels on steatotic hepatocytes.
Methods: Lipid accumulation in primary human hepatocytes was induced by incubation with the fatty acid oleate. Subsequently, steatotic and control hepatocytes were incubated with up to 50 mM alcohol.
Results: In this dose range, alcohol alone had only a minimal effect but further increased oleate induced lipogenesis and cellular triglyceride content, as well as lipid peroxidation and NFkappaB-mediated pro-inflammatory gene expression. Alcohol and steatosis also synergistically induced CYP2E1 expression and activity, whereas CYP2E1 inhibition blunted the joint pathological effects of alcohol and steatosis. Surprisingly, analysis of autophagy related genes, LC3 modification and p62 degradation revealed that alcohol induced autophagy in steatotic but not in control hepatocytes via synergistic activating effects on c-Jun N-terminal Kinase (JNK). Further induction of autophagy with rapamycin ameliorated the joint effect of alcohol and oleate stimulation on cellular lipid accumulation and inflammatory gene expression while inhibition of autophagy with 3-methyl adenine further enhanced the dual pathological effects.
Conclusions: Our model indicates that alcohol and steatosis synergistically induce pathological as well as protective mechanisms in hepatocytes *via* CYP2E1. These findings may have important implications for the prognosis and treatment of alcoholic liver disease particularly in obese individuals.

P1126
PHARMACOLOGICAL TREATMENTS FOR SEVERE ALCOHOLIC HEPATITIS: A NETWORK META-ANALYSIS

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Background and Aims: The optimal pharmaceutical treatment of severe alcoholic hepatitis remains controversial. We performed a systematic review and network meta-analysis of different pharmacological interventions for the treatment of such patients (DF >32 or hepatic encephalopathy).
Methods: We included randomized controlled trials (RCTs) of various pharmacological interventions compared with each other or with placebo. We conducted network meta-analyses following the National Institute for Health and Care Excellence Decision Support Unit guidance to compare multiple interventions simultaneously for each of the primary (mortality, adverse events, health-related quality of life) and secondary outcomes (cirrhosis, decompensation, HCC, liver transplantation) and only included trials in which participants could receive any of the interventions being assessed (transitivity assumption). We obtained a network plot to ensure that the trials are connected by treatments. We conducted a Bayesian network meta-analysis and calculated the odds ratios (OR) with 95% credible intervals (CrI) for assessing the effect of treatment.
Results: We included 21 RCTs on different treatment regimens of severe alcoholic hepatitis, comprising of 1765 patients.
The combination of steroids and N-acetylcysteine (NAC) was the most effective treatment in reducing 1 month mortality (20 trials; 1494 patients; OR = 0.12, 95%CrI 0.04–0.36) followed by steroids and pentoxifylline alone and the combination of the two (OR = 0.44, 95%CrI: 0.26–0.76; OR = 0.43, 95%CrI: 0.24–0.77; OR = 0.40, 95%CrI 0.17–0.90, respectively).
A total of 660 and 1299 patients included in 8 and 11 trials respectively were analyzed for 3-months and 12-months mortality. NAC plus steroids, and steroids and pentoxyfylline monotherapy were statistically significant compared to placebo for these periods. Pentoxyfylline showed the best efficacy in reducing episodes of liver decompensation (8 trials, 955 patients; OR = 0.26, 95%CrI 0.13–0.53) and liver-related mortality (12 trials, 797 patients; OR = 0.26, 95%CrI 0.13–0.51) compared to placebo. Nutritional therapy also showed a marked positive effect on one-year mortality (OR = 0.17, 95%CrI 0.06–0.52).
Conclusions: In patients with severe alcoholic hepatitis, the administration of steroids in combination with NAC, and steroids and pentoxyfylline alone or combined appear to reduce 1, 3 and 12-month mortality. Combining NAC to steroids should be further assessed by RCTs in such patients.

P1127
RESPONSE TO STEROID TREATMENT IN SEVERE ACUTE NON-ACETAMINOPHEN DRUG-INDUCED LIVER INJURY

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Background and Aims: Idiosyncratic drug-induced liver injury (DILI) is a leading cause of acute liver failure. It is still a matter of debate whether steroid treatment provides benefit for severe DILI and could prevent progression to liver failure.
Methods: All cases of non-acetaminophen DILI who were admitted to our department between 2010 and 2014 and who received a liver biopsy were analyzed retrospectively. Median initial dose of prednisolone treatment was 1 mg per kg body weight before steroids were tapered. Liver histologies were re-evaluated and the grade of hepatic inflammation was quantified by the modified Hepatic Activity Index (mHAI) score.
Results: 72 cases of DILI were analyzed. The most commonly implicated drugs were antibiotics (26.4%), phenprocoumon (15.3%) and non-steroidal anti-rheumatic drugs (13.9%). Most patients (71%) reached at least a DILIN severity score 3 (raised ALT or AP and total bilirubin level >2.5 mg/dl and hospitalization). 10 patients (13.9%) progressed to liver transplantation (LTX) or died (3 patients are still alive after LTX, 4 patients died after LTX and 3 patients were not eligible for LTX because of older age and comorbidities). These patients had a significantly higher initial MELD score than patients who recovered (32 vs 16; $p < 0.0001$). 60 patients (83%) received prednisolone treatment. Patients treated with prednisolone and an mHAI score ≥6 showed a significantly better response to treatment than patients with an mHAI score <6, measured by the decline of ALT from the day of starting prednisolone until day 3 (ΔALT 311 vs. 111 U/l; median reduction of 28.8% vs. 17.1%; $p = 0.04$) or measured by the decline of serum bilirubin from the day of starting prednisolone until week 4 (Δbilirubin 4.4 vs. 3.2 mg/dl; median reduction of 75.4% vs. 62.3%; $p = 0.02$). In prednisolone-treated patients who progressed to liver transplantation and/or died, serum bilirubin levels rose from the day of starting prednisolone until day 3 of treatment (Δbilirubin 0.7 mg/dl; median rise of 3.7%), whereas in patients under prednisolone who recovered, bilirubin levels fell (Δbilirubin 0.9 mg/dl; median reduction of 25%; $p = 0.02$).
Conclusions: Histological grading of liver inflammation could guide steroid treatment in severe DILI. A rise of bilirubin levels on day three after the start of steroids may indicate poor prognosis. Prospective studies are needed to evaluate if early steroid treatment can prevent fatal outcomes in severe DILI.

P1128

HEPATOCYTES DIFFERENTIATION FROM URINE CELLS DERIVED TRANSGENE FREE INDUCED PLURIPOTENT STEM CELLS

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Background and Aims: Reprogramming of somatic cells into pluripotent stem cells by using exogenous factors, hold great promises in development of personalized regenerative medicine and can produce valuable in vitro models of diseases. iPSCs have been generated from multiple sources including skin, extra embryonic tissues, which have their own advantages or disadvantages. The ideal cell source should be easily accessible, susceptible, and universal. Urine is the most easily accessible sample which can be collected from any individual. In the present study, we have generated iPSCs. from urine cells that has the potential to differentiate into hepatic lineages
Material and Methods: Urine samples were collected in sampling bottles. Exfoliated urine cells were isolated and cultured from adult human urine. After the appearance of colonies at 2–3 weeks, these cells were reprogrammed to iPSCs using commercially available "Keisuke Okita plasmid". Appeared stem cell like colonies were further characterized for stemness by studying the expression of pluripotent markers and by in-vitro tri-lineage differentiation.
Results: Stem cell like colonies generated from urine cells (urine iPSCs) on immunocytochemistry, were found to be positive for different pluripotent markers like OCT3/4, nanog, They also exhibited robust diffentation property when cultured as Embryoid bodies without growth factors. And differentiated in to hepatocytes like cells showing marker of Hepatocytes like albumin.
Conclusions: We document here for the first time, generation of transgene free urine cell derived induced pluripotent stem cells showing pluripotency. These UiPSCs are advantageous over from other sources as this process is purely non-invasive and generates transgene free iPSCs.

P1129

IDENTIFICATION OF PXR-HAPLOINSUFFICIENCY BY NEXT GENERATION SEQUENCING IN A PATIENT WITH ANABOLIC STEROID-INDUCED CHOLESTASIS

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Background and Aims: Use of anabolic steroids (AAS) is increasing among amateur athletes (Evans-Brown et al. *Lancet* 2008). Many AAS users report side effects, including severe cholestasis in selected cases. The lack of genome-wide association study signals for drug-induced liver injury (DILI) beside the known major histocompatibility complex risk alleles supports the concept that strong genetic determinants of DILI may be largely drug-specific and reflect rare genetic variations.
Methods: A 32-year-old Caucasian male with no significant medical history was admitted to our department with acute AAS-induced cholestasis. He had taken AAS for two months. Acute jaundice and pruritus developed two weeks after termination of AAS. Laboratory tests revealed increased serum bilirubin level but normal alkaline phosphatase and aminotransferase activities. There were no signs of biliary obstruction, and other viral and non-viral liver dieases were excluded. Liver biopsy showed blunt cholestasis. To investigate the genetic predisposition for this severe cholestatic phenotype, we performed targeted next generation sequencing (NGS: Illumina MiSeq) covering all coding sequences of selected genes (*ABCB4, ABCB11, ATP8B1, ABCC2, ABCG5/8, CIRH1A, CLDN1, JAG1, NOTCH2, NR1H4, NR1I2, VIPAR, VPS33B, UGT1A*) involved in hepatobiliary transport. This revealed the presence of a heterozygous 2 base pair deletion in exon 1 of the pregnane X receptor (PXR: nuclear receptor *NR1I2*). The mutation results in a frameshift (c.43_44del: p.R15fs), which causes complete loss of functional protein from the affected allele and thereby haploinsufficiency of PXR. Under therapy with prednisone, rifampin and ursodeoxycholic acid as well as albumin dialysis, pruritus and jaundice normalized, and the patient was discharged without any signs of persistent liver disease.
Conclusions: To our knowledge this is the first case report of a patient with inherited PXR insufficiency who developed acute AAS-induced cholestasis. Our results pinpoint the genetic predisposition to develop cholestasis after use of AAS, and further NGS-based studies are encouraged to determine the full spectrum of private mutations in nuclear receptor genes that increase the risk of drug-induced cholestasis.

P1130

ALCOHOLIC LIVER DISEASE: EFFECT OF HEPATOCYTES AND MACROPHAGES CO-CULTURE IN THE INFLAMMATORY RESPONSE

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Background and Aims: Macrophage migration Inhibitory Factor (MIF) is a pleiotropic cytokine involved in the pathogenesis of several acute and chronic inflammatory diseases, such as Alcoholic Liver Disease (ALD). Few information is available about MIF regulation between hepatocytes and macrophages in response to ethanol (EtOH). The aim of this work is to elucidate if the cross talk between these two cell types has any effect in the hepatic inflammatory response.
Methods: Co-culture (transwell system) of hepatocytes (HuH7) and differentiated macrophages (THP1+100nM PMA) were exposed for 24 h to 25mM EtOH; monocultures of each cell type were used as controls (CTRL). We assessed the gene expression of MIF and its receptor CD74 as well as TNF-α. In order to correlate gene expression with MIF functionality we also quantified the amount of released cytokine into the culture medium by ELISA (all data are reported in Table 1).

Table 1. Summary of the obtained data both in monoculture and co-culture system in response to ethanol exposure

	Folds (mean±SD)			
Cell type	MIF	CD74	TNF-α	MIF-release
Effect of 25mM EtOH in monoculture. Folds vs. untreated controls				
Hepatocytes	1.6±0.2*	1.0±0.1	2.6±1.1**	1.1±0.2
Macrophages	0.7±0.01	1.0±0.5	0.8±0.2	0.74±0.03
Effect of 25mM EtOH in co-culture. Folds vs. 25mM EtOH-treated monoculture				
Hepatocytes	1.12±0.43	5.54±0.18***	41.4±4.6***	3.09±1.3*
Macrophages	1.0±0.29	1.33±0.05*	0.25±0.06*	3.84±1.5*

*p < 0.05.; **p < 0.01; ***p < 0.001.

Results: In line with previously published data we confirmed that, in hepatocytes, EtOH induces the up-regulation of TNF-α. Furthermore we found that, in terms of gene expression, MIF follows the same trend of increase, even if the amount of released MIF was unchanged. Expression of CD74 was barely detectable in hepatocytes. Conversely, macrophages monocultures exposed to EtOH did not show any changes on the parameters under study.
Interestingly, we observed that in the co-culture system only hepatocytes presented a dramatic increase in TNF-α gene

expression, whereas macrophages presented a decrease. Moreover, in co-culture both hepatocytes and macrophages increased the release of MIF and with no changes at mRNA level. These data demonstrate that there is a synergic inflammatory response to EtOH when cells are cultured together. Furthermore, we observed a significant up-regulation of CD74 in co-cultured hepatocytes, which lead to the conclusion that cells are responding differently when macrophages are present in the system.

Conclusions: In conclusion, our data suggest that EtOH deleterious effect has hepatocytes as target cells. In addition, we showed that injured hepatocytes are able to modulate macrophages response, which (once activated) contribute to perpetrate the inflammatory state by increasing the production of MIF.

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P1131
AUTOIMMUNE HEPATITIS-LIKE DRUG INDUCED LIVER INJURY

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Background and Aims: Drug induced liver injury (DILI) can mimic many acute and chronic liver diseases including idiopathic autoimmune hepatitis (AIH). Hepatotoxic medications most commonly associated with an autoimmune presentation include nitrofurantoin, minocycline, methyldopa and hydralazine. The aim of this study was to characterize the clinical and serological features of DILI from these 4 agents.

Methods: Among over a thousand DILI cases enrolled in the U.S. Drug-Induced Liver Injury Network (DILIN) between 2004 and March 2014, 88 probable, highly likely and definite cases were included; 42 due to nitrofurantoin, 28 to minocycline, 11 to methyldopa and 7 to hydralazine. Available baseline (BL; n = 64) and follow up (FU; n = 39) sera were tested for antibodies to nuclear antigen (ANA), to Actin (AAA) and Soluble Liver Antigen (SLA) using the same ELISA kits as well as IgG levels. Genotype data on the AIH associated *HLA-DRB1*0301* and *HLA-DRB1*0401* alleles were available in 52 patients, which were compared with 4432 racially matched controls.

Results: The 88 cases included 80 women (91%) with a wide range in latency to onset (4 to 2162 days), predominantly hepatocellular injury (74%) and high rate of severe injury (25%). At BL, 46 of 64 patients (72%) tested positive for ANA, 39 (61%) for AAA, but none for SLA. IgG levels were elevated in 25 (39%) and were above 1.1 times ULN in 22 (25%). An autoimmune score (AIS) was derived from the degree of elevation in IgG, ANA, AAA or SLA (each as 0, 1+ or 2+). Autoimmune features (score ≥2) were more common with nitrofurantoin (82%) and minocycline (73%) than methyldopa (55%) or hydralazine (43%) (P = 0.16). FU specimens showed a decline in IgG levels and loss of ANA reactivity in 30% and AAA in 35% of initially positive subjects as well as a decrease in AIS (BL: 3, range 0–6, vs FU: 2, range 0–4; P < 0.001). These changes were not associated with corticosteroid therapy, which was used in 58% in this cohort. The *HLA-DRB1*0301* (15%) and *HLA-DRB1*0401* (9%) alleles were not overrepresented in this cohort when compared to controls (13% and 10% respectively). In addition, no patient was homozygous for these alleles and there was no association with autoimmune phenotype.

Feature	Nitrofurantoin	Minocycline	Methyldopa	Hydralazine
n	42	28	11	7
Female gender (%)	42 (100)	22 (79)	11 (100)	5 (71)
Latency to onset, (days), median (range)	561 (4–2162)	294 (29–1350)	81 (17–238)	108 (13–208)
Hepatocellular, n (%)	28 (67)	23 (82)	11 (100)	3 (43)
Severe, n (%)	11 (26)	2 (7)	2 (18)	1 (14)
Fatal, n (%)	5 (12)	0 (0)	0 (0)	1 (14)
Baseline serum sample available, n	28	19	10	7
ANA +, n (%)	23 (79)	15 (79)	4 (40)	4 (57)
AAA +, n (%)	23 (79)	10 (53)	4 (40)	2 (29)
IgG >ULN	12 (43)	6 (32)	5 (50)	2 (29)
Autoimmune phenotype (≥2), n (%)	23 (82)	14 (74)	6 (60)	3 (43)

Conclusions: Autoimmune like DILI occurs in most but not all patients with nitrofurantoin and minocycline liver injury and in at least half of those with methyldopa and hydralazine injury. The autoimmune phenotype decreases with recovery and is not associated with the typical HLA alleles found in idiopathic AIH.

P1132
REGULATION OF OXIDATIVE STRESS AND LIPID PEROXIDATION IN METHOTREXATE-INDUCED LIVER TOXICITY BY PRETREATMENT WITH TURMERIC

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Background and Aims: Methotrexate (MTX) is an antimetabolite compound, which is broadly used in treatment of cancer and autoimmune diseases. MTX-induced hepatotoxicity limits its clinical application. Turmeric (TUR) contains various chemical constituents, has marked potential for preventing and treatment of several disorders. We investigated the efficacy of turmeric against hepatotoxicity caused by MTX.

Methods: All experiments were performed on male Wistar albino rats that were randomly divided into six groups. Group one received saline orally for 30 days (control group), groups two and three received turmeric extract (100 mg/kg, 200 mg/kg respectively) orally for 30 days, group four received single dose, of MTX IP at day 30, groups five and six received turmeric extract (100 mg/kg) and (200 mg/kg) orally respectively for 30 days and single dose of methoterxate IP (20 mg/kg) at day 30. Four days after MTX injection animals were sacrificed and evaluated. Blood ALT and AST (markers for hepatocyte injury), ALP and bilirubin (sensitive markers of biliary function), albumin (reflect liver synthetic function) as well as the plasma TAS concentration (marker of antioxidant defenses) were determined. The cellular antioxidant defense activities were examined in liver tissue samples using SOD, CAT, and GSH-Px for the oxidative stress, and MDA for lipid peroxidation. In addition, liver damage was evaluated by histopathology.

Results: MTX significantly induced liver damage (P < 0.05) and decreased its antioxidant capacity while turmeric was hepatoprotective. Liver tissue microscopic evaluation showed that MTX treatment induced severe centrilobular and periportal degeneration, hyperemia of portal vein, increased artery inflammatory cells infiltration and necrosis, while all of histopathological changes were attenuated by turmeric (200 mg/kg).

Conclusions: Thus, pre-administration of turmeric extract can successfully attenuate MTX hepatotoxicity and improve the therapeutic index of MTX.

P1133

HEPATIC VENO-OCCLUSIVE DISEASE (SINUSOIDAL OBSTRUCTION SYNDROME) AFTER SOLID ORGAN VERSUS HEMATOPOIETIC STEM CELL TRANSPLANTATION: ARE THESE DIFFERENT DISEASE ENTITIES?

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Background and Aims: The term sinusoidal obstruction syndrome (SOS) has replaced veno-occlusive disease (VOD) to describe hepatic venous outflow obstruction related to liver microvascular injury, since the target of injury is believed to be endothelial cells in the hepatic sinusoids, as demonstrated in animal models. Classically, SOS is described after hematopoietic stem cell transplantation (HCT), but it can also occur after solid organ transplantation (SOT). At our center, we have noted that patients with SOS/VOD in the context of SOT do not present with the classic acute syndrome as in HCT. The purpose of this study was to investigate the clinical, laboratory and histopathologic characteristics of HCT and SOT patients developing SOS/VOD to determine if these may be distinct disease entities.
Methods: Retrospective case series of HCT and SOT patients with a diagnosis of SOS/VOD at the University Health Network. All patients who had a clinical diagnosis of SOS/VOD and underwent liver biopsy were included. Patients with clinical and/or radiologic features of heart failure or large hepatic vein/IVC disease were excluded. Data including clinical presentation, medical history and laboratory parameters was collected and histopathology slides were reviewed. Descriptive data are presented.
Results: Between August 2001 and August 2014, five SOT (2 liver, 3 kidney) and three HCT patients met the study criteria. Mean time to liver biopsy was 45.6 days after HCT (range 30–60 days) and 8.3 years after SOT (range 1–14 years). HCT patients presented with a clinical syndrome of portal hypertension and liver dysfunction while SOT patients presented with asymptomatic ALP elevation. 1/5 SOT patients had exposure to azathioprine. Ascites was present in all HCT and none of the SOT patients. Mean bilirubin was 20.2 umol/L in the SOT group and 176.7umol/L in the HCT group. Liver histopathology of HCT cases revealed marked sinusoidal congestion, no fibrosis, and often patent hepatic venules. In SOT, histopathology revealed mild congestion, subsinusoidal fibrosis, and lost or remodeled hepatic venules.
Conclusions: SOS/VOD following SOT presents distinctly from that after HCT. Patients present late with asymptomatic ALP elevation without jaundice or ascites and have histological evidence of fibrosis and small vein remodeling. These findings raise the possibility that these may be distinct disease entities but further study is needed.

P1134

THE LEUKOCYTE MIGRATION INHIBITOR SOLUBLE CD18 INCREASES WITH SEVERITY OF HUMAN ALCOHOLIC HEPATITIS

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Background and Aims: The TNFα levels and macrophage activity markers such as soluble CD163 (sCD163) are very high in human alcoholic hepatitis. The β2 (CD18) family of integrins is shed from the surface of myeloid cells in response to TNFα forming the recently discovered soluble CD18 (sCD18), which binds to endothelial adhesion molecules. This is thought to inhibit extravasation of leukocytes and facilitate their efflux from tissues. It is not known whether this tissue inflammation moderating mechanism is intact in alcoholic hepatitis. We, therefore, measured the plasma levels of sCD18 in these patients.
Methods: We analysed blood samples from 50 patients with severe alcoholic hepatitis at diagnosis, at day 14 and at day 30 and from 20 healthy controls with in-house immunosorbant assays for sCD18 and sCD163.
Results: At the diagnosis of alcoholic hepatitis, the sCD18 concentration was increased by 30% in the patients compared with controls (median±IQR; 1907.3±1032 vs. 1472.5±646.2, $p = 0.01$). This did not change during the follow-up period. We found a positive correlation between sCD18 and sCD163 ($r = 0.53$ $p = 0.0001$). The levels of sCD18 correlated with disease severity according to the Child Pugh ($r = 0.41$ $p = 0.003$) and Glasgow Alcoholic Hepatitis Score ($r = 0.30$ $p = 0.04$).
Conclusions: The tissue inflammation-limiting shedding of sCD18 is induced in patients with alcoholic hepatitis in parallel with macrophage activation and disease severity, suggesting an anti-inflammatory mechanism that is operative within the picture of florid hepatic inflammation.

P1135

UTILITY OF LIVER BIOPSY IN ALCOHOLIC HEPATITIS: DATA FROM THE STEROIDS OR PENTOXYFILLINE IN ALCOHOLIC HEPATITIS (STOPAH) CLINICAL TRIAL

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Background and Aims: The role of liver biopsy in the diagnosis of alcoholic hepatitis (AH) remains controversial. We sought to investigate this in patients recruited to *Steroids or Pentoxyfilline for Alcoholic Hepatitis* (STOPAH).
Methods: Patients with clinically diagnosed severe AH [Maddrey's discriminant function (mDF) >32] were recruited and liver biopsies were obtained either before or after administration of trial medication. Two independent histopathologists, blinded to treatment group and patient outcome, scored histological features of AH, including those described in the Alcoholic Hepatitis Histological (AHHS) and Kleiner NAFLD Activity (NAS) scores.
Results: 208 biopsies were received (19% of patients recruited to STOPAH). 93 samples were both of adequate quality and taken between admission and day 5 of treatment. Baseline serum markers of severe disease were associated with histological features of severe disease (e.g. serum bilirubin and bilirubinostasis, white cell count and biopsy inflammation; see Table 1).
Overall, 82/93 (88%) samples had histological features consistent with alcoholic steatohepatitis (ASH). In 59 cases data regarding indication for biopsy was available: 35/59 were obtained as routine clinical practice without diagnostic uncertainty. In these patients 31/35 (91%) biopsies were diagnostic for ASH. The 24/59 patients biopsied for diagnostic uncertainty had a lower biopsy diagnosis rate, 20/24 (83%) had histological features consistent with ASH.
69/82 cases with a diagnosis of ASH, had complete follow up data. In this cohort mortality at day 28 was 13% and 22% at 90 days. 42 (61%) patients were in the severe AHHS category and 46 (67%) were in the highest NAS category. Patients in the AHHS severe category had a significantly increased day 28 mortality rate (21% vs. 0%, Kruskal–Wallis $p = 0.01$). However there was no significant different at day 90 day or when using the NAS scoring system.
AHHS categorisation positively correlated with mDF and Glasgow Alcoholic Hepatitis Score (GAHS), however it did not correlate with

other clinical scores or predictors of outcome (see Table 1). There was no association between NAS categorisation and any clinical parameters (see Table 1).

Table 1

Clinical marker	Comment	Statisitical test, value
93 cases with biopsy taken between admission and day five of treatment		
Serum PT ratio	Higher PT ratio associated with advanced cirrhosis	
	Laennec classification:	Kruskal–Wallis, p = 0.01
	STOPAH classification:	Kruskal–Wallis, p = 0.01
Serum INR	Higher INR associated with advanced cirrhosis	
	Laennec classification:	Kruskal–Wallis, p = 0.01
Serum AST	Higher AST associated with more advanced steatohepatitis	
	Kleiner NAS classification:	Kruskal–Wallis, p = 0.03
Serum bilirubin	Higher bilirubin associated with more advanced steatohepatitis	
	AHHS classification:	Kruskal–Wallis, p = 0.003
	Kleiner NAS classification:	Kruskal–Wallis, p = 0.04
	Higher bilirubin associated with more biopsy hepatocellular bilirubinostasis	Kruskal–Wallis, p ≤ 0.0001
Serum white cell count	Higher white cell count associated with more severe lobular inflammation	
	Kleiner NAS classification:	Kruskal–Wallis, p = 0.001
	STOPAH classification:	Mann–Whitney U, p ≤ 0.0001
Serum neutrophil count	Higher neutrophil count associated with more severe biopsy PMN infiltrate	
	AHHS classification:	Kruskal–Wallis, p = 0.001
	STOPAH classification:	Mann–Whitney U, p ≤ 0.0001
69 cases from cohort above taken as routine clinical practice and with a histological diagnosis of steatohepatitis		
mDF	Maddrey's Discriminant Function	
	AHHS classification:	r_S = 0.223, p = 0.045
	Kleiner NAS classification:	r_S = 0.008, p = 0.940
GAHS	Glasgow Alcoholic Hepatitis Score	
	AHHS classification:	r_S = 0.300, p = 0.009
	Kleiner NAS classification:	r_S = 0.219, p = 0.060
MELD	Model of End-Stage Liver Disease	
	AHHS classification:	r_S = 0.175, p = 0.115
	Kleiner NAS classification:	r_S = 0.020, p = 0.860
Lille score	0.45 cut off	
	AHHS classification:	Kruskal–Wallis p = 0.107
	Kleiner NAS classification:	Kruskal–Wallis p = 0.574
Day 7 change in bilirubin	AHHS classification:	Kruskal–Wallis p = 0.371
	Kleiner NAS classification	Kruskal–Wallis p = 0.422

Conclusions: The findings demonstrate parallels between clinical and histological parameters and support the role of liver biopsy in cases of diagnostic uncertainty. Further work into the role of liver biopsy in predicting response to therapy in ASH is warranted.

P1136

MOST LIVER RELATED DEATHS ARE CAUSED BY ALCOHOL: AN AUDIT OF LIVER MORTALITY FROM TERTIARY CARE CENTER IN NORTH INDIA

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Background and Aims: Alcohol, hepatotropic viruses, and non-alcoholic steatohepatitis (NASH) are the most important causes of liver related deaths. The distribution of these causes among Indian patients dying of liver disease is unknown. This information will help in prioritizing healthcare efforts in preventing liver related deaths in India.

Methods: Records of all consecutive patients who had died due to liver-related causes in the Gastroenterology Department of Sir Ganga Ram Hospital, New Delhi, India, from Nov 2010 to Oct 2014 were analyzed. Patients dying due to non-hepatic cancers metastasizing to liver were excluded. Clinical presentation and immediate etiological causes of death were analyzed. In patients with multiple factors, the most immediate etiology was taken as the cause of death.

Results: Records of 401 consecutive patients were analyzed. Nine patients were excluded who had died of liver metastasis from non-hepatic cancers; hence 392 patients were included in the study (median age 50 [range 14–87] years, males 80%). Underlying chronic liver disease (CLD) was present in 86% (335/392) while in 14% (57/392) there was no underlying CLD. In CLD group most patients (71%, 237/335) had presented with complications of cirrhosis (such as end-stage liver disease, portal hypertension, sepsis etc.). Acute-on-chronic liver failure was the presentation in 29% (98/335) of CLD patients. Among patients without underlying CLD the most common presentation was with acute liver failure (68%, 39/57). Overall, the most common cause of liver-related death was alcohol, responsible for 30% (118/392) of deaths, followed by NASH/cryptogenic in 23% (91/392), hepatotropic viruses in 19% (73/392), bacterial/other infections in 12% (45/392), and drug-induced liver injury in 6% (24/392). In 5% cases the cause was some unidentified acute hepatic insult. The distribution of causes of death in various presentations is shown in the table.

Conclusions: Alcohol is the most important cause of liver related deaths in India and NASH/cryptogenic cirrhosis is the second important cause. Since, both these causes are preventable by increasing public awareness and implementing life-style measures, urgent attention should be paid towards these measures.

Table (abstract P1136).

	All patients (n = 392)	Underlying CLD (n = 335)		Non-CLD (n = 57)		
		Cirrhosis complications (n = 237)	ACLF (n = 98)	ALF (n = 39)	Acute intrahepatic cholestasis (n = 10)	Liver abscess (n = 8)
Alcohol	118 (30%)	79 (33%)	39 (40%)	–	–	–
Hepatotropic viruses	73 (19%)	42 (18%)	15 (15%)	16 (41%)	–	–
Bacteria, non-hepatotropic viruses, protozoa	45 (12%)	8 (3%)	19 (19%)	–	10 (100%)	8 (100%)
NASH or cryptogenic	91 (23%)	91 (38%)	–	–	–	–
Unidentified acute liver injury	18 (5%)	–	8 (8%)	10 (26%)	–	–
Drug-induced liver injury	24 (6%)	–	16 (16%)	8 (20%)	–	–
Miscellaneous	23 (6%)	17 (7%)	1 (1%)	5 (13%)	–	–

Autoimmune and chronic cholestatic liver disease

P1137
INTEGRATED EFFICACY SUMMARY FOR OBETICHOLIC ACID IN SUBJECTS WITH PRIMARY BILIARY CIRRHOSIS

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Background and Aims: Obeticholic acid (OCA), a modified bile acid and farnesoid x receptor (FXR) agonist, is currently in late-phase development for the treatment of primary biliary cirrhosis (PBC), a rare cholestatic liver disease. In two Phase 2 trials (3 months) and in the Phase 3 POISE trial (12 months) 5 mg to 50 mg OCA, once daily as monotherapy or add-on to UDCA, produced significant improvements in markers of cholestasis and inflammation. The objective of this integrated analysis was to evaluate the efficacy of ≤10 mg OCA, the proposed indicated doses for the treatment of PBC, as monotherapy or in combination with UDCA in an integrated fashion across the 3 trials compared to placebo.
Methods: Individual subject data from 3 completed, randomized, controlled double-blind trials of OCA once daily were pooled for this analysis (OCA ≤10 mg N = 201; placebo N = 134; OCA 25 mg N = 48; OCA 50 mg N = 57). All placebo subjects were pooled as were OCA doses of 5 mg, titration from 5 to 10 mg, and 10 mg (≤10 mg OCA). Inclusion criteria included ALP ≥1.67×ULN or total bilirubin >ULN but <2×ULN. Analyses were repeated based on baseline concomitant UDCA use. Efficacy was evaluated using a composite endpoint shown to correlate with long-term survival (ALP <1.67 ULN with a minimum 15% reduction and a normal bilirubin) (Lammers 2014) and assessment of liver biochemistry and inflammation.
Results: Baseline characteristics were generally similar between treatment groups. Baseline ALP was higher in monotherapy subjects compared to UDCA combination subjects. Efficacy data are presented in the Table. At the end of double blind treatment significantly more OCA-treated subjects achieved the composite endpoint compared to placebo when used as monotherapy or in combination with UDCA. OCA treatment was associated with statistically significant and clinically meaningful decreases in ALP as well as GGT, ALT, and AST. While the subjects in the monotherapy group had higher ALP at baseline than the OCA plus UDCA group, the two groups reached similar ALP levels by the end of the double-blind period. Pruritus was the most common adverse event associated with PBC and showed a dose-related increase in incidence with OCA.
Conclusions: In this pooled analysis, a significantly greater percentage of subjects with PBC treated with OCA with or without UDCA achieved a composite endpoint shown to correlate with long-term survival compared to placebo-treated subjects.

P1138
LONG-TERM OUTCOME OF PATIENTS WITH AUTOIMMUNE HEPATITIS RECEIVING MYCOPHENOLATE MOFETIL (MMF) AS FIRST LINE TREATMENT

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Background and Aims: The natural history of autoimmune hepatitis (AIH) depends vastly on treatment. We have previously shown, that using MMF as first line therapy instead of conventional treatment with corticosteroids ± azathioprine, results in high rates of remission with fewer side effects, quick corticosteroid withdrawal and 0% non-response rate. In the present study we investigated the long term outcome of patients with AIH receiving MMF as front line treatment, with special emphasis on outcome after treatment withdrawal.
Methods: One hundred and five patients with well defined AIH [follow-up for 66 (3–168) months] were treated with prednisolone 1 mg/kg/day plus 1.5–2 g/day MMF. Treatment outcomes were defined according to International Autoimmune Hepatitis Group and AASLD guidelines. Patients were candidates for MMF withdrawal after being on treatment for at least 4 years and having complete response (CR) at least the last 2 years.
Results: 99/105 (94%) responded initially to treatment normalizing the aminotransferases and γ-globulins within 2 (1–18) months: 77 (73.3%) had CR [52/77 (67.5%) maintained the CR after prednisolone withdrawal], 22/77 (21%) achieved initial response but they relapsed during prednisolone withdrawal and 6/77 (5.7%) had partial response (PR). No patient was non-responder. Predictors of CR were acute disease presentation (p = 0.003) and shorter treatment duration (p = 0.01). None of the 80 non-cirrhotic patients developed cirrhosis during follow-up [63 (3–143) months]. 7/25 cirrhotic patients decompensated (28%), 4 died of liver related causes, 1 was transplanted and 4 developed HCC. So far, treatment was stopped in 31/105 patients after 60 (12–125) months. 23/31 (74%) remained in remission for 21 (3–84) months; 8/31 relapsed in 3.5 (2–7) months. Maintenance of response was associated with absence of cirrhosis at diagnosis (p = 0.05), shorter corticosteroid duration (p = 0.009), lower IgG levels on month 1 (p = 0.016) and 6 of treatment (p = 0.013). In multivariate analysis, no parameter could independently predict maintenance of remission after treatment withdrawal.
Conclusions: So far, MMF proved to be an efficient and safe first line treatment for AIH achieving the highest rates of maintenance of remission off treatment (74%) ever published though the remission

Table (abstract P1137).

	Monotherapy						Concomitant UDCA					
	Placebo (N=28)			≤10 mg OCA (N=31)			Placebo (N=106)			≤10 mg OCA (N=170)		
Composite Endpoint	End of DB	CMH p-value		End of DB	CMH p-value		End of DB	CMH p-value		End of DB	CMH p-value	
Percent response	4%			35%	0.0019		9%			48%	<0.0001	
	Base	End of DB	Δ	Base	End of DB	Δ	Base	End of DB	Δ	Base	End of DB	Δ
ALP, U/L	401 (207)	415 (235)	−45 (27)	451 (262)	254 (149)	−231 (26)***	307 (113)	300 (136)	11 (8)	308 (110)	208 (83)	−83 (7)***
Total bilirubin, μmol/L	11.4 (6.5)	12.9 (9.3)	−2.3 (1.1)	12.7 (8.0)	12.2 (7.9)	−4.4 (1.1)*	11.7 (6.9)	12.2 (8.0)	1.2 (0.7)	11.5 (6.7)	10.6 (6.5)	−1.0 (0.6)**
GGT, U/L	495 (367)	535 (455)	−48 (44)	564 (339)	186 (184)	−427 (42)***	251 (361)	250 (322)	14 (12)	242 (191)	105 (101)	−128 (10)***
ALT, U/L	80 (55)	80 (64)	11 (7)	84 (50)	50 (37)	−22 (7)***	52 (29)	49 (27)	1 (2)	56 (35)	36 (27)	−15 (2)**
AST, U/L	70 (36)	68 (45)	7 (6)	69 (37)	53 (35)	−7 (6)*	46 (23)	47 (34)	6 (2)	42 (20)	38 (34)	−6 (2)***

Baseline (Base) and End of Double-blind (DB) data are mean (SD); Δ = LS mean change from Baseline (SE); Composite endpoint analyzed with Cochrane–Mantel–Haenszel (CMH) test; P values for comparing OCA to placebo: *p < 0.05, **p < 0.002, ***p < 0.0001.

criteria used were strict. As relapse after treatment withdrawal is almost universal with conventional therapy, MMF seems a reasonable first line treatment of AIH.

P1139

TESTOSTERONE SUPPRESSES HEPATIC INFLAMMATION BY THE DOWN-REGULATION OF IL-17, CXCL-9 AND CXCL-10 IN A MOUSE MODEL OF EXPERIMENTAL CHOLANGITIS

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Background and Aims: Most autoimmune liver diseases predominantly affect women. Here we aimed to elucidate how sex affects autoimmune hepatic inflammation in a novel and inducible mouse model of autoimmune cholangitis, which showed female preponderance similar to human autoimmune diseases.
Methods: Adoptive cell transfer of ovalbumin (OVA)-specific CD8+ T cells into mice expressing OVA peptide under control of the human keratin 14 promoter were performed.
Results: OT1–1 CD8+ T cell transfer resulted in portal inflammation and cholangitis in female, but not in male recipient mice. Recruitment of transferred CD45.1+CD8+ T cells into the livers of recipient male or female mice was similar. In contrast, the recruitment of endogenous CD45.2+CD4+ T cells into female livers was increased compared to male livers indicating a pro-inflammatory role for endogenous CD4+ T cells. Transfer of CD4+ T cells isolated from inflamed livers of K14-OVAp mice could cause liver inflammation in K14-OVAp recipient mice in the absence of OT-1 cells. Increased CD4+ T cell numbers were associated with increased frequencies of CD4+IL-17+ T cells, higher amounts of RORc mRNA and significantly higher production of IL-17 by hepatic nonparenchymal cells (NPCS) isolated from female compared to male mice [220.8 pg/ml female vs. 57.5 pg/ml male, $p < 0.017$]. Moreover, levels of T cell recruiting chemokines CCL-9 and -10 were increased in female livers. Cell transfer experiments into K14-OVApxIL17a/f ko female recipient mice confirmed that lack of endogenous IL-17 significantly decreased ALT levels and cholangitis severity compared to K14-OVAp IL-17 wild-type mice. IL-17 expression, CCL-9 and -10 expression and CD4+ T cell recruitment could be modulated by castration and hormone replacement studies. Male resistance to liver inflammation could be attributed to the protective effects of testosterone, which was shown to act directly on CD4+ T cells by suppressing TH17 differentiation in vitro
Conclusions: Testosterone was found to be the major determinant of the observed sexual dimorphism in our cholangitis model. IL-17 production by CD4+ T cells seems to increase cholangitis severity and may be modulated by testosterone directly. These results should stimulate future investigations of the influence of sex hormones on liver inflammation and the gender differences observed in human autoimmune liver diseases.

P1140

NGM282 DEMONSTRATES POTENT ANTIFIBROTIC ACTIVITY IN A MOUSE MODEL OF SEVERE CHOLESTATIC FIBROSIS

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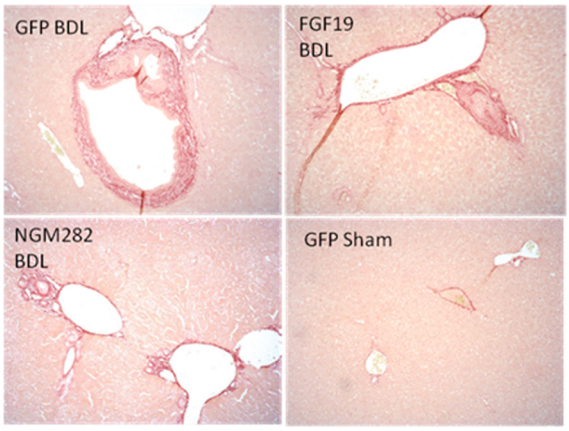
Background and Aims: NGM282 is an engineered recombinant protein variant of fibroblast growth factor 19 (FGF19) that retains CYP7a1 inhibition *via* FGFR4–b klotho binding but lacks the abnormal proliferative activity of FGF19. We have previously shown that NGM282 is hepatoprotective from cholestatic injury compared to obeticholic acid and bezafibrate in mice (Luo 2014). In the current study, we evaluated the anti-fibrotic effects of FGF19 and NGM282 in a mouse model of cholestatic fibrosis.
Methods: Cholestatic fibrosis was induced by bile duct ligation (BDL) in male FVB mice. Adeno-associated virus encoding green fluorescent protein (GFP) (as control), FGF19 or NGM282 were injected intravenously into mice *via* tail vein at 4 days before BDL surgery. Mice were euthanized 28 days after the BDL. Serum chemistry, liver histopathology and fibrosis gene expression analyses were conducted.
Results: FGF19 and NGM282 significantly reduced the Sirius red staining areas indicating reduction of fibrosis compared to GFP control (Figure 1). Additionally, fibrotic gene expression of Col1a, Ctgf, Tgfb1 and Timp1was suppressed by both of the molecules. Both FGF19 and NGM282 showed significantly less dilatation of bile ducts, vein and lymphatics and reduced bile duct proliferation and inflammation in H&E staining compared to untreated animals. Serum liver enzymes, bilirubin, total bile acids and survival rates were also substantially improved with the treatments.
Conclusions: Both FGF19 and NGM282 effectively prevented the animals from hepatic fibrosis and the associated pathological changes in a mouse model of cholestatic fibrosis as well as improving the biochemical markers of cholestasis. Suppression of hepatic bile acids synthesis may represent a novel therapy for the treatment and prevention of cholestatic fibrosis. We are actively pursuing the development of NGM282 in the clinical setting.



P1141

POST-TRANSLATIONAL REGULATION OF POLYCYSTIN 2 (PC2) EXPRESSION AS A NOVEL MECHANISM OF CHOLANGIOCYTE REACTION TO BILIARY DAMAGE AND REPAIR

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Background and Aims: In Polycystic Liver Diseases, defective PC2 leads to elevated production of cAMP, PKA-dependent activation of the ERK1/2 pathway, HIF1α-mediated VEGF production and stimulation of cyst growth and progression. Activation of the ERK/HIF1α/VEGF pathway in cholangiocytes is fundamental during repair from biliary damage. In this study we hypothesized that PC-2 levels can be modulated during biliary damage/repair, resulting in activation of the ERK/HIF1α/VEGF pathway.
Methods and Results: PC2 protein expression was significantly reduced in livers from mice undergoing cholangiocyte damage

(Mdr2$^{-/-}$-KO, bile duct ligation, treatment with dehydrocollidine – DDC). However, PC-2-gene expression was not reduced, suggesting that the decrease in PC2 protein content was due to increased degradation. To understand if factors involved in biliary damage influence PC2 protein expression, mouse cholangiocytes were treated with pro-inflammatory cytokines (IL-1β, IFNγ, TNFα), nitric oxide (NO) donors (DETAnonoate) and ER stressors (thapsigargin). Expression of PC2 protein was again significantly reduced, but not its gene expression. Noteworthy, downregulation of PC-2 was associated to increased ERK1/2 phosphorylation, HIF1α transcription activity, secretion of VEGF and phosphorylation of VEGFR2. Expression of Herp and NEK, ubiquitin-like proteins that promote PC2 degradation by increasing the retrotranslocation from the ER to the proteasome complex was also increased. Pre-treatment with the proteasome inhibitor MG-132 restored the expression of PC2 in cells treated with cytokines but not in cells treated with NO donors or with ER stressors. In the latter conditions, PC2 degradation was inhibited by LY294002, an inhibitor of PI3K that blocks the autophagosome formation and by blockade of lysosomes with chloroquine, suggesting a role for autophagy. Finally, treatment of DDC-treated mice with the proteasome inhibitor bortezomib, significantly reduced the extent of the liver damage and restored PC2 expression.

Conclusions: PC2 protein is modulated post-translationally by pro-inflammatory cytokines, ER-stressors and NO-donors and is reduced in mice with biliary damage. Down regulation of PC2 protein expression in cholangiocytes increases the ERK1/2/HIF1α pathway and VEGF secretion, thereby playing a pivotal role in the regulation of cholangiocyte response to biliary damage. Treatments able to restore PC2 expression (i.e. proteasome inhibitors) may represent a new therapeutic approach in biliary diseases.

P1142
NEXT GENERATION SEQUENCING ANALYSIS OF CHOLESTATIC PATIENTS REVEALS HIGH IMPACT VARIATION IN COMMON AND RARE CANDIDATE GENES: DISSECTING THE GENETIC ARCHITECTURE OF CHOLESTASIS

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Background and Aims: Cholestasis indicates bile secretory failure, which might be caused by environmental factors and gene variants, or combination of both. Although there is variability in underlying causes of hepatocellular cholestasis, selected patients present with chronic cholestasis of unknown etiology, and gene mutations of critical hepatobiliary transporters or their regulators may be suspected. Our *aim* now was to employ next-generation sequencing (NGS) to dissect genetic risk factors in adult patients with cholestatic liver disease of unknown etiology.

Methods: The full length coding sequences of a panel of genes known to be involved in regulation and transport of drugs and metabolites into bile were determined by NGS sequencing in patients with unexplained chronic cholestasis, as defined by an at least two-fold increase of serum alkaline phosphatase (AP) activities and ALT/AP ratio ≤2. Population frequency and biochemical impact of all detected variants were analysed. Rare, high-impact variants were also considered. Cholestasis NGS gene panel: *ABCB4, ABCB11, ABCC2, ABCG5, ABCG8, ATP8B1, CIRH1A, CLDN1, JAG1, NOTCH2, NR1H4, NR1I2, UGT1A1–10, VIPAR, VPS33B.*

Results: Full sequence analysis of 24 genes in 22 patients with cholestatic liver disease revealed heterozygosity for several high-impact variants that have previously been associated with cholestatic liver disease such as *ABCB11* p.G982R (PFIC2), *ABCB11* p.D482G (PFIC2), *ATP8B1* p.I661T (PFIC1/BRIC), *ATP8B1* p.D70N (BRIC/ICP) and *VPS33B* c.177+1G>A (ARC). New high-impact variants that are highly likely to result in non-functional protein (complete loss of splice site, frameshift deletion) were detected in *ABCB4 (MDR3)*, *NR1I2* (*PXR*) and *UGT1A7*. None of these variants were found among 62 000 individual sequences in the Exome Aggregation Consortium (ExAC) database. Rare, non-conservative variants of yet unknown biochemical impact detected in *ABCC2* (*MRP2*), *NOTCH2* and *NR1H4* (*FXR*) will also be presented.

Conclusions: Next-generation sequencing permits rapid and comprehensive analysis of a large panel of cholestasis-associated transcripts. Peculiar phenotypes appear more likely to result in the detection of novel pathogenic mutations. Correlating the results with systematic clinical phenotyping might result in improved genotype-phenotype correlations and lead to targeted chemical intervention such as chaperone-therapy.

P1143
THYROID HORMONE RECEPTOR BETA 1 (THRβ1) STIMULATES BILIARY PHOSPHATIDYLCHOLINE SECRETION VIA ABCB4 REGULATION – A NEW THERAPEUTIC APPROACH FOR CHOLANGIOPATHIES

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Background and Aims: ATP-binding cassette protein subfamily B4/multidrug resistance transporter 3 (ABCB4/MDR3) mediates phosphatidylcholine (PC) biliary secretion. Since PC is essential for formation of mixed micelles counterating bile acid (BA) toxicity, defects in ABCB4 expression result in bile duct and cholestatic liver injury. Conversely up-regulating *ABCB4* pharmacologically may improve cholestatic liver and bile duct injury in cholangiopathies (e.g. PBC and PSC).

We investigated the role of triiodothyronine (T3) in *ABCB4* regulation in vitro (primary immortalized human cells) and in vivo (mice).

Methods: HepG2, IHH and HuH7 cells were incubated with T3 and specific THR alpha (THRa) and beta (THRb) agonists. Mice were fed a liver specific THRb agonist KB141, before assessment of bile composition/flow. Hepatobilary transporter/BA synthetic enzyme gene expressions was measured by Q-PCR.

Results: *ABCB4* gene expression and protein levels were increased in a time- and dose-dependent manner by T3 as well as liver specific THRb (GC1, KB2115, CGS23425 and KB141) but not THRa agonists in all human cell lines (up to 2 times; $p < 0.05$ after 24 h). In vitro actinomycin D, but not cycloheximide, treatment abolished *ABCB4* up-regulation by T3 revealing a direct transcriptional regulation. ABCB4 promoter in silico analysis mapped 7 potential THR response elements, but gel shifts assays identified only one THRb specific site 6kb upstream to the transcription start site. Interestingly, co-incubation of T3 and FXR agonists resulted in additive stimulation of ABCB4 up to 3 fold $p < 0.05$, while repressing CYP7A1 expression. Moreover, *Abcb4* was up-regulated (1.5 fold $p < 0.05$) in wild-type mice treated with KB141 which in turn increased biliary PC secretion 5 fold.

Conclusions: We identified THRβ1 as a novel activator of *ABCB4* in human and mouse. Moreover, FXR and THRb agonists may synergistically modify bile composition by increasing PC and reducing BA thus favouring less toxic bile and opening new therapeutic opportunities in cholangiopathies such as PBC and PSC.

P1144

HEPATOCYTE SPECIFIC EXPRESSION OF A DOMINANT STABLE FORM OF BETA-CATENIN RESULTS IN CHOLESTATIC LIVER DISEASE

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Background and Aims: The Wnt/β-catenin signaling pathway is an ancient, evolutionary preserved systems, which plays a crucial role in embryonic development, tissue homeostasis, wound healing and malignant transformation in a variety of different organs, including the liver. Continuous Wnt/β-catenin signaling and somatic mutations in exon 3 of the *ctnnb1* gene are observed in >30% of patients with liver cancer. To investigate the role of continuous β-catenin signaling in liver biology, we generated mice, which lack exon 3 of the *ctnnb1* gene specifically in hepatocytes.
Methods: Mice with a loxP flanked exon 3 of the *ctnnb1* gene were crossed to albumin-Cre or SA-CreERT2 mice to obtain mice with hepatocyte specific expression of a dominant stable form of β-catenin (catn ex3Δ hep mice). Catn ex3Δ hep mice were analyzed by serum biochemistry, histology, Sirius red staining, immunoblotting, immunohistochemstry, immunofluorescene, confocal microscopy, electron microscopy and mRNA profiling.
Results: Successful removal of exon 3 of the *ctnnb1* gene was confirmed by immunoblotting using whole liver tissue. Mice were born at the expected Mendelian ratio. $Ctnnb1^{CAhep}$ mice were smaller and their body weight was significantly reduced. Livers of $Ctnnb1^{CAhep}$ mice were significantly enlarged, differed in macroscopic appearance and the liver to body weight ratio was significantly increased. Serum ALT, AST and bile acid levels were significantly increased in $Ctnnb1^{CAhep}$ mice. Livers of $Ctnnb1^{CAhep}$ mice displayed a disturbed liver architecture, paucity of central veins, mitotic figures and proliferating cholangiocytes. Sirius red and desmin staining indicated a biliary type of fibrosis. $Ctnnb1^{CAhep}$ mice did not differ from Cre-negative controls with regards to expression of bsep as evaluated by qPCR, but expression of compensatory bile acid transporters including abcb4, mdr1a and mrp2 were significantly increased. $Ctnnb1^{CAhep}$ mice also displayed significantly reduced mRNA levels of ntcp but significantly increased levels of mrp4. mRNA data was confirmed at the protein level by immunoblotting. Expression of cyp7a1 the rate-limiting step in bile acid synthesis was significantly increased in $Ctnnb1^{CAhep}$ mice. In contrast, expression of cyp27, cyp8b1 and cyp2b10 was reduced in $Ctnnb1^{CAhep}$ mice. Similar results were obtained when exon 3 was removed in adult animals.
Conclusions: Expression of a dominant stable form of β-catenin in hepatocytes results in severe cholestasis and biliary type fibrosis.

P1145

ACTIVATION AND EPITHELIAL-TO-MESENCHYMAL TRANSITION OF BILIARY TREE STEM CELLS WITHIN PERIBILIARY GLANDS ARE INVOLVED IN THE PATHOGENESIS OF PRIMARY SCLEROSING CHOLANGITIS

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Background and Aims: Primary sclerosing cholangitis (PSC) is characterized by fibrotic strictures of the extra-hepatic (EHBD) and/or intra-hepatic bile ducts (IHBD) leading to progressive cholestatic liver injury. The involvement of biliary tree stem cell (BTSC) compartment in the pathogenesis of PSC is virtually unknown. We investigated the BTSC compartment located in peribiliary glands (PBGs) of EHBDs and large IHBDs of human liver and its role in the development of the characteristic fibrosing PSC lesions.
Methods: Liver specimens containing EHBDs or large IHBDs were obtained from patients with PSC at different disease stages (n = 11) and, as controls, from normal livers (n = 6) and patients with primary biliary cirrhosis (n = 6). Specimens were processed for histology, immunohistochemistry and immunofluorescence.
Results: PSC is characterized by hyperplasia and mucinous metaplasia of PBGs that correlate with progression of the disease. PBG hyperplasia participates together with inflammation and fibrosis in determining the wall thickening of EHBD or large IHBD and is associated with the occurrence of dysplastic lesions. The increase of PBG mass is determined by the expansion of the BTSC transit-amplifying compartment, which sprout towards surface epithelium. In PSC, BTSCs and myofibroblasts generally display marked up-regulation of Hedgehog pathway markers. In ducts with onion-skin-like fibrosis, PBG cells express traits of epithelial-to-mesenchymal transition (EMT) co-localized with markers of Hedgehog pathway up-regulation and cellular senescence. PBGs were minimally affected in PBC.
Conclusions: BTSCs within PBGs are activated in the course of PSC, their proliferation being sustained by up-regulation of the Hedgehog pathway. BTSCs participate in the wall thickening of bile ducts and contribute, via EMT, to onion-skin-like fibrosis and thus the focal distribution of these lesions. PBG hyperplasia was associated with the occurrence of dysplasia in PSC and this could be involved in the development of cholangiocarcinoma in this disease.

P1146

RECURRENCE OF PSC AFTER LIVER TRANSPLANTATION IN THE NORDIC COUNTRIES

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Background and Aims: Recurrence of primary sclerosing cholangitis (rPSC) has been reported to occur in about 20% of all

transplanted PSC patients. A number of riskfactors for recurrence have been described, but few findings have been consistent. In this study we aimed to identify and confirm riskfactors for rPSC in a large cohort of liver transplanted PSC patients.

Methods: All liver transplanted PSC patients in the Nordic countries (Denmark, Sweden, Norway, Finland) between 1984 and 2006 (n = 440) were included in the analysis. The diagnosis of recurrent PSC (rPSC) was based upon the Graziadei criteria. Risk factors for recurrence were assessed using a specific protocol, the Nordic liver transplant registry and a previous study database with extensive IBD data including immunosuppression, medical and surgical treatment for IBD. Survival data was analyzed using the Kaplan–Meier method, and risk factors for recurrence analyzed with uni- and multivariate Cox-regression analysis.

Results: We were able to include 313 patients in the final analysis; patients with no liver biopsy or cholangiography following transplantation (n = 70) were excluded from the study. For the survival analysis patients with follow up <6 months (n = 31) or cholangiocarcinoma in the explant (n = 26) were excluded. Patients with rPSC (n = 82; 26%) were significantly older than patients without rPSC (n = 231). The only risk factor associated to recurrence of PSC was age at transplantation; an intact colon did not influence the risk of developing rPSC, there was no difference in terms of gender, IBD or type of treatment after OLT. Patients with an intact colon at the time of transplantation had a significantly better survival (p = 0.04 Log Rank).

Conclusions: Recurrent PSC in the Nordic countries is more common than previously reported, with a rate of 26%. We found that age at transplantation is a risk factor for development of recurrent disease. An intact colon at transplantation is associated with a better long-term survival.

P1147

VALIDATION OF AN ALKALINE PHOSPHATASE AND BILIRUBIN RESPONSE CRITERION AS BIOMARKER FOR TRANSPLANT-FREE SURVIVAL IN PRIMARY BILIARY CIRRHOSIS IN THE WORLD'S TWO LARGEST COHORTS

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Background and Aims: Serum alkaline phosphatase (ALP) and bilirubin levels are characteristically elevated in primary biliary cirrhosis (PBC) and decrease of these levels is associated with improved prognosis. Therefore the criterion ALP <1.67×ULN together with bilirubin <1×ULN has recently been used as primary endpoint for evaluation of treatment of PBC patients in randomized controlled trials. However, validation of this response criterion is lacking. In the world's two largest independent PBC cohorts, from the national UK-PBC and the international Global PBC Study Group, we aimed to validate the use of ALP <1.67×ULN and bilirubin <1×ULN as a response criterion associated with improved prognosis.

Methods: Both cohorts are observational with long-term follow-up: the UK-PBC data is collected prospectively. The Global PBC data is a meta-analysis of individual patient data from established cohorts from 15 sites in 8 countries. The response criterion, ALP <1.67×ULN and bilirubin <1×ULN, was studied with Cox regression analyses in different settings, stratified by center and adjusted for age, sex, calendar time and use of ursodeoxycholic acid (UDCA). The endpoint was defined as liver transplantation (LTx) or liver-related death.

Results: Data from 4022 and 4845 PBC patients were included in this analysis. Median follow up time was 6.6 years (IQR, 3.3–11.1) in the UK-PBC cohort and 7.3 (IQR: 3.5–11.5) in the Global PBC cohort. 537 and 747 patients, respectively, reached the endpoint of LTx or liver-related death, in the two cohorts. In both cohorts the response criterion at 1-year follow up was

strongly associated with the endpoints (table), irrespective of sub-population. Especially for the sub-population including patients with an elevated bilirubin >1×ULN or an elevated ALP >1.67 at baseline, but with bilirubin <2×ULN, the HR of non-response versus response was above 3 and strongly significant. The analyses were repeated at different follow-up time points (2–5 years) in the Global PBC cohort and similarly strong associations were found.

Table: Hazard ratio (HR) for the endpoint: LT and liver related death of non-response versus response at 1 year follow-up

Population	UK-PBC				Global PBC			
	N	HR	95%CI	p-value	N	HR	95%CI	p-value
Total study population	4022	7.9	5.9–10.7	<0.0001	4845	4.2	3.3–5.2	<0.0001
UDCA treated	2775	10.7	6.9–16.8	<0.0001	4119	4.3	3.4–5.5	<0.0001
Untreated	712	6.2	4.1–9.3	<0.0001	640	3.3	1.6–6.2	0.0007
Male	335	2.7	1.18–6.3	0.012	497	2.4	1.3–4.6	0.008
Female	3152	8.9	6.5–12.4	<0.0001	4348	4.4	3.4–5.6	<0.0001
Age <55 years	1634	8.9	5.8–13.7	<0.0001	2431	4.7	3.3–6.6	<0.0001
Diagnose year <1990	139	6.1	2.6–14.2	<0.0001	1013	3.5	2.3–5.3	<0.0001
Diagnose year 1990–1999	815	9.8	6.0–16.1	<0.0001	1813	4.4	3.2–6.0	<0.0001
Diagnose year 2000–2010	2499	12.1	6.6–22.2	<0.0001	1916	5.4	3.2–9.2	<0.0001
Biopsy: no cirrhosis	320	3.8	1.3–11.1	0.014	1378	7.2	3.5–15.0	<0.0001
Biopsy: possible cirrhosis	168	5.4	1.9–15.4	0.002	638	2.7	1.7–4.2	<0.0001
Bilirubin <2 & (Bili >1 or ALP >1.67)	2201	5.1	3.6–7.1	<0.0001	2066	3.5	2.4–5.3	<0.0001

Conclusions: This uniquely powered, international collaboration confirms the use of ALP and bilirubin as a response criterion that is strongly associated with LTx and liver-related death.

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LONG-TERM ADMINISTRATION OF URSODEOXYCHOLIC ACID PREVENTS RECURRENCE OF PRIMARY BILIARY CIRRHOSIS AFTER LIVER TRANSPLANTATION

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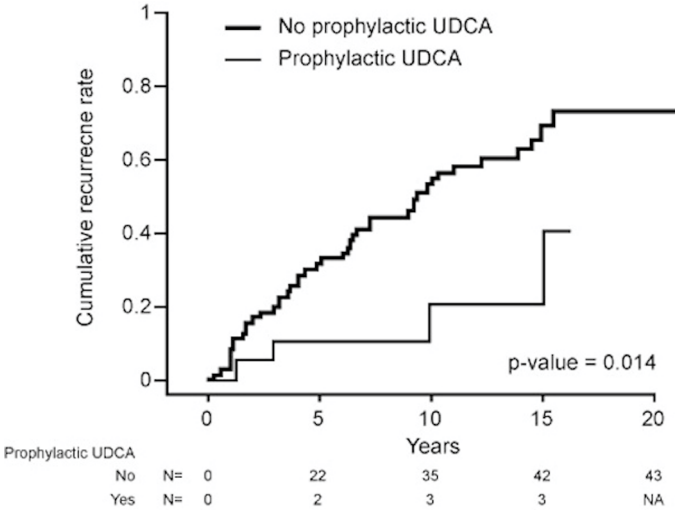
Background and Aims: Recurrence of primary biliary cirrhosis (PBC) after liver transplantation (LT) is not uncommon and can occasionally lead to severe graft dysfunction and retransplantation. Ursodeoxycholic acid (UDCA) is a safe and effective treatment for PBC. The aim of this study was to determine whether routine administration of UDCA after LT could affect the incidence of PBC recurrence.
Methods: All patients who had undergone LT for PBC in 5 French and Swiss centers from 1988 to 2010 were retrospectively studied. A minimal follow-up of one year after LT was required to be included. Recurrences of PBC were biopsy-proven. In all centers, biopsies were routinely performed at 1, 5, 10, 15, 20 and 25 years of follow-up, and at any time when clinically indicated. The patients from a single French center were administered UDCA (13–15 mg/kg/d) in routine as a non-specific protective measure started at the time of LT. The patients from the other centers were treated with UDCA only in case of proven recurrence.
Results: A total of 90 patients (women: 86%; mean age at LT: 54 years) fulfilled inclusion criteria, including 19 (21%) who were receiving UDCA since LT. The mean follow-up was 12 yrs. Recurrence was diagnosed in 48 (53%) patients. The mean number of biopsies and the mean follow-up did not differ according to the recurrence or UDCA statuses. The median time for recurrence was 6 yrs. Cumulative incidence of recurrence was 27%, 47%, 61%, and 68% at 5, 10, 15, and 20 years, respectively. The risk of recurrence differed neither in recipient and donor characteristics nor in calcineurin inhibitor (cyclosporine vs. tacrolimus) regimens. In univariate and multivariate Cox models, routine administration of UDCA was the only factor that significantly affected the risk of recurrence (HR: 0.31; 95% CI: 0.11–0.85; p = 0.014; figure). Although 7 (15%) patients with recurrence progressed to graft cirrhosis, requiring retransplantation in one, neither recurrence nor routine administration of UDCA had a significant impact on survival.
Conclusions: This study strongly suggests that routine administration of UDCA after LT could prevent recurrence of PBC. This supports the extended use of UDCA as prophylaxis for PBC recurrence after LT.



P1149

PURIFIED MESENCHYMAL STROMAL CELLS REDUCE LIVER DAMAGE IN AN ALLO-IMMUNE MODEL OF LIVER DAMAGE (OVA-BIL)

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Background and Aims: The ability of mesenchymal stromal cells (MSC) to immumomodulate may have clinical relevance in immune-mediated liver damage. Inherent heterogeneity of unsorted MSC populations and impact of prolonged passage in culture may explain varied/reduced function in other settings. We set out to study the biology of a purified MSC population and impact of cytokine priming on their function.
Methods: FACS sorted MSC (PDGFRa Sca-1$^+$; PaS) were isolated from bone marrow (BM) of C57Bl/6 & BALB/c mice and their proliferation, karyotyping and trilineage differentiation studied over passage. This included *in vitro* (suppression of proliferation of co-cultured stimulated CD4$^+$CD25$^-$ T cells) and *in vivo* immunomodulation

studies (Ova-Bil model of allo-immune liver injury). Impact of cytokine priming (FGF2, $TGFb_1$, PDGF-BB) on action of PaS cells was studied.

Results: Medium passage (P3–5) PαS cells cultured in standard media differentiate to bone, cartilage and fat *in vitro* and reduce liver damage in the Ova-Bil model. Cells demonstrated colony forming capacity (CFU-F)/proliferation, although this slowed over passage with expression of senescence-associated β-galactosidase (from <3% to >40%). Simultaneously, late passage cells lost ability of adipogenic/chondrogenic differentiation, ability to suppress T cell proliferation *in vitro* and ability to reduce liver damage in the Ova-Bil model (Figure 1). To overcome this PαS cells were primed with cytokines known to govern MSC proliferation and differentiation (FGF2, PDGF-BB or $TGFβ_1$). Microarray analysis revealed marked differential upregulation of key adipogenic, chondrogenic and osteogenic transcripts between groups. Proliferation of PαS cells was higher with FGF, PDGF, and $TGFb_1$. $TGFb_1$ supplemented cells had attenuated tri-lineage differentiation, but displayed a prolonged ability to immunosuppress *in vitro* and *in vivo* compared with untreated cells (Figure 1). Numbers of liver-infiltrating CD4 and CD8 cells (of both donor derived OT1/OT2 and recipient origin) were reduced after infusion of MSC primed with $TGFb_1$. Detailed karyotypic analyses revealed no significant abnormalities of treated cells.

Conclusions: Purified (PαS) MSC are potent immunomodulators, although in keeping with unsorted MSC, function is lost with passage. Indiscriminate priming with cytokines can reduce immunomodulatory function of MSC, with only $TGFb_1$prolonging their immunosuppressive function in a model of allo-immune liver damage.

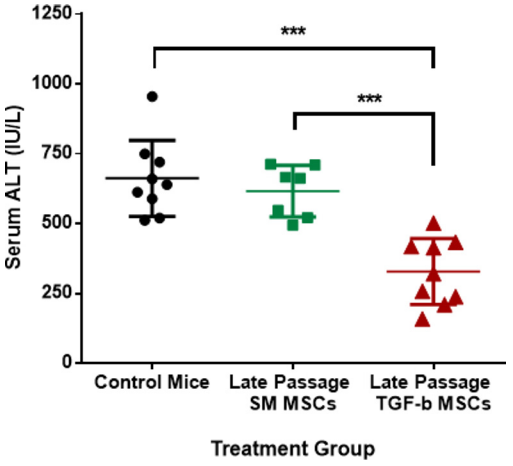

Figure 1.

P1150
CHARACTERIZATION AND OUTCOME OF 93 PATIENTS WITH ISCHEMIC-LIKE CHOLANGIOPATHY WITH SECONDARY SCLEROSING CHOLANGITIS

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Background and Aims: Sclerosing cholangitis in critically ill patients (SC-CIP) with sepsis and ARDS is a cholestatic liver disease, which is characterized by progressive sclerosis of the biliary tree, biliary casts and infection. Until now, little is known about the risk factors and outcome of SC-CIP patients.

Methods: At the University Hospital of Regensburg medical records of 93 patients (f=16, m=77) with SC-CIP were evaluated (between 2002 and 2014). The following parameters were analyzed: cause of sepsis, duration of mechanical ventilation, duration of ICU treatment, age at diagnosis of SC-CIP, microbiology results, time point of diagnosis by ERCP, body weight, survival data, cause of death, and other parameters.

Results: In all patients SC-CIP was diagnosed by ERCP and 81% of patients had Enterococcus in the bile. Age at SC-CIP diagnosis was 56+15 years. Causes of sepsis were polytrauma (28%), post-operative complications (28%) or internal morbidities (44%). The duration on the intensive care unit (ICU) was 48±30 days, and duration of respiratory ventilation was 32±19 days. 46% of patients were temporary on hemodialysis. The mean follow-up was 30±39 months. Twenty-four patients underwent liver transplantation. The 1- and 5-year-survival of all SC-CIP patients was 48% and 34%, respectively. Causes of death were: sepsis (79%), cardiac insufficiency (7%), malignancy (7%), and hemorrhagic shock (7%).

Conclusions: SC-CIP must be kept in mind in icteric patients with sepsis and long-term ICU therapy. ERCP is the gold standard for diagnosis of SC-CIP. The prognosis of SC-CIP is unfavourable. LT is the only curative treatment of SC-CIP.

P1151
GENETIC VARIANTS OF UDP-GLUCURONOSYLTRANSFERASE (UGT) 1A GENES MODIFY THE PRESENTATION AND DISEASE PROGRESSION IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS

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Background and Aims: Primary sclerosing cholangitis (PSC) is a chronic progressive cholestatic liver disease with unknown pathogenesis and without a curative medical therapy. The human UDP-glucuronosyltransferases 1A (UGT1A) play a major role in the detoxification and elimination of bilirubin, bile acids and a number of xenobiotics linked to inflammation and tumorigenesis. The relationship between genetic *UGT1A* variants and the course and outcome of PSC has not yet been analyzed.

Methods: A large cohort of German PSC patients with a long-term-follow-up was genotyped for *UGT1A* variants (SNPs) including UGT1A1*28, UGT1A3 −66 T>C, and UGT1A7 p.N129K/p.R131K usingTaqMan-5′-nuclease-assays. The results were correlated with clinical characteristic and the transplant-free survival of the patients.

Results: 328 patients with well characterized and documented PSC were included into the study (70.1% male, mean age at diagnosis 32.6 years, 69.1% with IBD). The mean transplant free survival time was 15.6 years. Patients with wild-type alleles of all three *UGT1A* genes (n = 44) had a significantly longer transplant free survival (19.6 years vs. 14.3 years, p = 0.042, log-rank-test) than patients carrying a homozygous or heterozygous SNP variant in at least one of the *UGT1A1*, *UGT1A3* or *UGT1A7* genes (n = 284). Additionally, we found that the patients carrying wild-type alleles of all three *UGT1A* genes had a lower serum-bilirubin (25 vs. 38 μmol/l, p = 0.023) and serum-cholesterol (195 vs. 223 mg/dl, p = 0.033) at first presentation. Furthermore inflammatory bowel disease was found to be associated with wild-type UGT1A alleles (81.8% vs. 67.1%, p = 0.0499).

Conclusions: This large cohort shows an association with SNP variants of the *UGT1A1, UGT1A3* and *UGT1A7* genes and outcome in PSC. A recently described *UGT1A* variant haplotype, present as a homozygous trait in 10% of the population and leading to impaired glucuronidation of xenobiotics capable of driving inflammatory reactions and carcinogenesis is implicated as a risk factor for the progression of PSC. Higher bilirubin levels may be associated with UGT1A1*28 alleles responsible for Gilbert's syndrome. Thus, *UGT1A*

variants may represent a tool for the prognostic stratification of PSC patients and link disease progression in PSC to the regulation of detoxification by glucuronidation.

P1152

A COMPARATIVE STUDY OF PRURITUS IN PBC COHORTS FROM UK, USA AND ITALY

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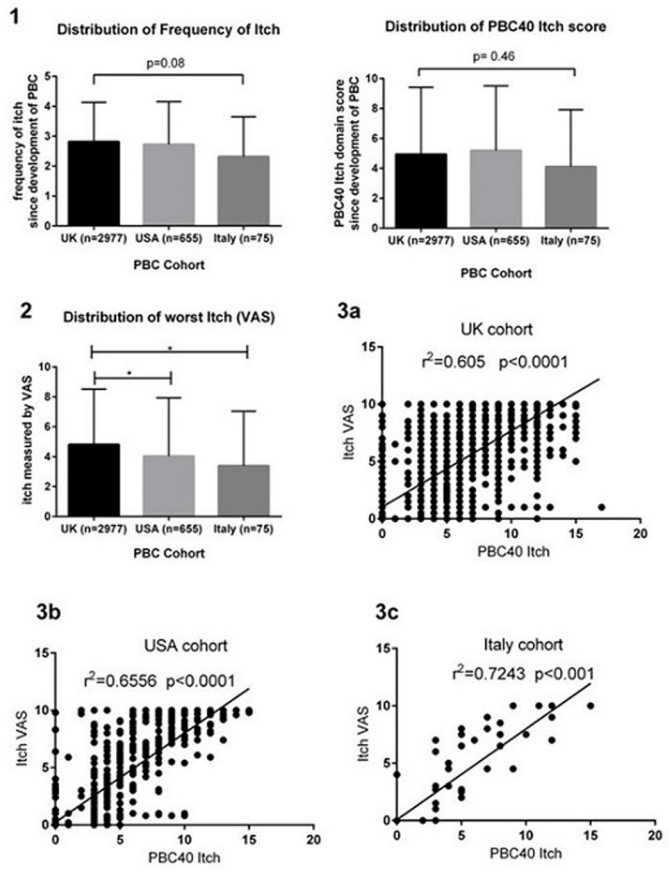
Background and Aims: Pruritus (itch) is a common problem in cholestatic liver diseases such as Primary Biliary Cirrhosis (PBC). There are limited studies of 'real-life' experience of cholestatic itch and its treatment in international cohorts. Colestyramine is the recommended first line therapy for cholestatic pruritus. We aimed to compare pruritus experienced by PBC patients from UK, USA and Italy to: (1) understand the prevalence of pruritus in PBC in each cohort, (2) report the frequency of anti-pruritic treatments received by PBC patients from their local health care, (3) study any differences in the frequency and intensity of pruritus between the cohorts and (4) assess the correlation between measures of itch intensity in each cohort.
Methods: This was a cross-sectional study of experience of itch reported by PBC patients without a liver transplant from UK, Mayo clinic (USA) and Italy. Data were collected on frequency of itch (never, rarely, occasionally, frequently, all the time), PBC-40 itch score, intensity of worst itch measured using a 0–10 visual analogue scale (VAS) and treatment received for their itch since diagnosis of PBC. We defined persistent itch as pruritus occurring 'frequently' or 'all the time' and severe itch as persistent itch with a combination of itch score of more than 8 on VAS.
Results: Data were available for 2977 PBC patients from UK, 655 from USA and 75 patients from Italy. 2076 (70%) UK, 445 (68%) USA and 45 (60%) Italy patients had experienced itch at some point in their illness. Of these, persistent itch was reported by 32% (UK), 34% (USA) and 27% (Italy) patients and severe itch by 15% of UK & USA and 13% of Italy patients. Patients with severe itch in UK, USA and Italy reported to have received treatment with colestyramine in 50%, 35% and 30% cases and rifampicin in 16%, 18% and 20% cases respectively. Figures 1 and 2 show the main results of comparison of cohorts ($^*p < 0.05$) and Figures 3a–c show significant correlation between measures of itch intensity in each cohort.
Conclusions: This comparative study of three independent international PBC cohorts suggests prevalence of pruritus in PBC is 60% to 70% of which nearly a third suffer with persistent itch and up to 15% experience severe itch. UK patients had higher scores for severity of worst itch since development of PBC. Treatment of itch in PBC patients appeared unsatisfactory in all three cohorts as more than half of patients with severe itch had not received the first line therapy.



P1153

ACUTE AUTOIMMUNE HEPATITIS INCREASES SECONDARY BILE ACIDS RESULTING IN INTESTINAL APOPTOSIS, INFLAMMATION, AND MUCOSITIS IN GROUP VIA CALCIUM-INDEPENDENT PHOSPHOLIPASE A2 KNOCKOUT MICE

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Background and Aims: Chronic bowel disease can co-exist with severe autoimmune hepatitis (AIH) in the absence of PSC. Genetic background may contribute to these overlap syndromes. We previously have shown that the deficiency of group VIA iPLA2 (iPLA2β) causes an accumulation of hepatocyte apoptosis, and renders the susceptibility for endotoxin acute liver injury. We here tested whether AIH induction in the liver in iPLA2β-deficient mice could result in intestinal injury, and whether bile acid toxicity could be involved.
Methods: Control (WT) and iPLA2$\beta^{-/-}$ female mice at 12 months old were intravenously injected with 10 mg/kg concanavalinA (ConA) or saline for 24 h. A profile of 15 bile acids was measured by LC/MS-MS. Functional assays included liver enzymes, Western blot analysis, qRT-PCR, histology and immunohistochemistry.
Results: Whole-body deficiency of iPLA2β led to a complete absence of this gene in liver and intestine. ConA treatment of iPLA2$\beta^{-/-}$ mice markedly increased liver enzymes, hepatic necrosis, fibrosis with α-smooth muscle actin activation, and biliary ductal reaction in a greater extent than that of treated WT mice. This was concomitant with increases of toxic secondary bile acids including lithocholic, deoxycholic, and chenodeoxycholic acids in liver. Interestingly, the levels of all bile acids were increased and decreased, respectively, in vena cava blood and jejunum of ConA-treated mutants compared with treated WT. While not affecting the duodenum of WT mice, ConA treatment of the mutant mice caused severe villous shortening and atrophy which was associated with increased apoptosis and the proliferation marker Ki67 on periportal lymphocytes. With a greater extent than in WT, ConA treatment of mutant mice increased jejunal expression of pro-inflammatory cytokines TNF-α, IL-6, and SOCS3 as well as chemokines CCL2 and

the receptor of CCL3, CCR5. The observed increased inflammation was concomitant with increased number of mucin-containing goblet cells in the duodenum and ileum, and this was associated with increased expression of a regulator of mucositis Krüppel-like factor 4.

Conclusions: iPLA2β deficiency renders susceptibility for AIH assoicated with increases of hepatic secondary bile acids which could induce intestinal mucosal damage, and hepatic and intestinal damage could in turn lead to an elevation of bile acids in the periphery. Thus, our experimental model demonstrates that AIH in a susceptible host such as with iPLA2β deficiency may lead to enteropathy via bile acid toxicity.

P1154

PRE-TREATMENT SERUM VITAMIN D STATUS IS ASSOCIATED WITH SUBSEQUENT RESPONSE TO UDCA IN PBC

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Background and Aims: Vitamin D is always linked with primary biliary cirrhosis (PBC) for bone complication in the patients. However increasing evidences suggest a non-skeletal role of vitamin D in various autoimmune or liver diseases. We performed this study to investigate the clinical relevance of vitamin D level in PBC, especially the association with therapeutic effects of ursodeoxycholic acid (UDCA).

Methods: Consecutive PBC patients were retrospectively studied. 25-hydroxyvitamin D [25(OH)D] levels were determined by pre-treatment frozen serum samples. Response to UDCA was evaluated by Paris-I and Barcelona criteria respectively. Logistic regressions were performed to identify treatment response associated parameters.

Results: Of 98 patients, the mean serum 25(OH)D concentration was 17.9±7.6 ng/mL. 25(OH)D levels decreased with increasing histological stage ($p = 0.029$) and negatively correlated with bilirubin, alkaline phosphatase and Mayo risk score. After one year of UDCA therapy, 31 patients failed to achieve complete response according to Paris-I criteria. Baseline 25(OH)D level was significantly lower in non-responders (14.8±6.4 vs. 19.3±7.6 ng/mL, $p = 0.005$). In multivariate analysis, vitamin D deficiency at baseline increased the risk of incomplete response, independently from elevated bilirubin and ALP values and advanced stages (OR = 3.93, 95% CI=1.02–15.19, $p = 0.047$). Similar results were obtained when biochemical response was evaluated by Barcelona criteria.

Conclusions: 25(OH)D level is associated with biochemical and histological features in PBC. Pre-treatment vitamin D status independently related to subsequent response to UDCA. Our results suggest adequate 25(OH)D levels may be helpful to improve response to UDCA and supplementation of vitamin D is worth considered, not only for preventing osteoporosis in the patients.

P1155

FXR AGONISM WITH OBETICHOLIC ACID MAY ATTENUATE BONE MINERAL DENSITY DECREASE IN SUBJECTS WITH PRIMARY BILIARY CIRRHOSIS

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Background and Aims: Osteoporosis occurs frequently in subjects with primary biliary cirrhosis (PBC). The farnesoid X receptor (FXR) expressed in bone has been shown to positively regulate bone osteogenesis in mice (Cho 2013). Obeticholic acid (OCA), a farnesoid x receptor agonist being developed for the treatment of PBC, produced significant improvements in indices of hepatic damage and function as well as markers of inflammation and cholestasis in the phase 3 POISE trial. This analysis evaluated the effect of OCA compared to placebo on bone mineral density (BMD).

Methods: Subjects with PBC ± UDCA (if taking UDCA patients were maintained on a stable dose) with ALP ≥1.67×ULN or bilirubin <2×ULN were randomized to PBO, OCA 5 or 10 mg for 12 months. Subjects on 5 mg were titrated to 10 mg after 6 months (OCA Titration) as clinically indicated. Dual-emission X-ray absorptiometry (DEXA) scan was used to assess BMD in a subset of subjects prior to and following 12 months of OCA or placebo treatment. Results of the femoral neck and lumbar spine (using T-score, Z-score, and BMD) were summarized. Changes from baseline at Month 12 were analyzed using an ANCOVA model with baseline values as a covariate. Osteopenia and osteoporosis were based on WHO thresholds: T score −1.0 to −2.5 and ≤−2.5, respectively.

Results: Of 216 subjects enrolled in the trial, 122 had DEXA scans at baseline and Month 12 (85% female; 22% ≥65 years of age; 52% postmenopausal). Baseline ALP was 318±102 U/L and 91% of subjects took concomitant UDCA. At baseline the prevalence of osteopenia and osteoporosis was 7% and 54%, respectively. Placebo subjects had a significant decrease in femoral T-scores ($p = 0.03$) and a reduction was also detected for 10 mg OCA ($p = 0.01$). OCA-treated subjects had significantly smaller decreases in femoral T-scores relative to placebo ($p < 0.05$). No significant differences from baseline or between treatment groups were seen in Lumbar BMD. Results were generally consistent but did not attain statistical significance when assessed based on menopausal status.

Conclusions: Individuals with PBC are at increased risk for osteopenia, osteoporosis and bone fractures. This preliminary analysis of BMD in subjects treated with OCA suggests that OCA may attenuate the deterioration in femoral T scores in subjects with PBC and merits further study of the effects of this drug in preventing bone loss and its complications.

Table (abstract P1155).

BMD	Placebo			Titration OCA			10 mg OCA		
	BL	End of DB	Δ	BL	End of DB	Δ	BL	End of DB	Δ
Lumbar BMD									
L2–L4 (g/cm^2)	0.97 (0.17)	0.97 (0.18)	−0.01 (0.01)	1.02 (0.19)	1.01 (0.20)	−0.01 (0.01)	1.03 (0.17)	1.00 (0.18)	−0.01 (0.01)
T score	−1.16 (1.47)	−1.42 (1.38)	−0.26 (0.14)	−1.10 (1.51)	−1.10 (1.65)	−0.01 (0.14)	−0.82 (1.30)	−1.02 (1.30)	−0.09 (0.14)
Femoral BMD									
Neck (g/cm^2)	0.79 (0.13)	0.76 (0.13)	−0.02 (0.01)	0.80 (0.12)	0.81 (0.13)	−0.01 (0.01)	0.87 (0.16)	0.81 (0.15)	−0.04 (0.01)
T score	−1.15 (1.17)	−1.48 (1.04)	−0.33 (0.11)†	−1.29 (0.95)	−1.28 (0.95)	−0.06 (0.11)*	−0.89 (1.04)	−1.06 (0.82)	−0.07 (0.11)†*

BMD, Bone mineral density.
Baseline (BL) and End of Double-blind (DB) values are mean (SD); changes from baseline values are LS mean (SE).
*$p < 0.05$ comparing OCA to placebo using an ANCOVA model; $^{\dagger}p < 0.03$ end of DB vs BL.

P1156

PROGNOSTIC VALUE OF LIVER HISTOLOGY IN AUTOIMMUNE HEPATITIS

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Background and Aims: Liver byopsy is recommended at diagnosis of autoimmune hepatitis and during follow-up. The data on the prognostic value of baseline liver biopsy and the sequential histology is either controversial or scanty.
The aims of the study are to evaluate the prognostic value of liver biopsy at time of diagnosis and during the disease at follow-up of autoimmune hepatitis.
Methods: All 98 patients from our hospital registry, with probable or definite autoimmune hepatitis from years 1995–2012 were studied. 66 percent had multiple liver biopsies and total follow-up was 818 patient years. A multivariate analysis was performed in order to find significant parameters which could predict patient survival, progression of fibrosis, and development of histological or clinical cirrhosis.
Results: At time of diagnosis, only seven percent of patients were cirrhotic. 52 percent of patients had concomitant autoimmune diseases. The only histological findings that predicted fibrosis progression were the total inflammation score (OR 6.22, CI 1.21–31.87, p = 0.028) and eosinophilic infiltration (OR 4.64, CI 1.08–20.01, p = 0.040). Women had an increased risk for fibrosis progression (OR 6.1, CI 1.2–30.8, p = 0.029). Risk for cirrhosis development was lower in men, HR 0.07, CI 0.01–0.42, p = 0.004. Patients with fibrosis stage 3–4 had remarkable greater risk for developing clinical cirrhosis than patients with fibrosis 1 and 2, HR 53.65, CI 7.49–384.18, p = 0.001 vs HR 6.84, CI 1.16–40.32, p = 0.03 and HR 3.39, CI 0.62–18.49, p = 0.018, respectively. Total inflammation (HR 64.2, CI 3.8–1082.7, p = 0.004), rosette formation (HR 8.03, CI 1.89–34.16, p = 0.005), and acute cholestasis (8.74, CI 1.73–44.09, p = 0.009) at baseline liver histology predicted development of cirrhosis. Amount of necrosis had no impact on fibrosis or cirrhosis progression. None of the patients with histological pericholangitis or granulomas developed cirrhosis and only one patient had progressive fibrosis during follow-up.
Conclusions: The baseline liver histology at diagnosis provides important prognostic information of cirrhosis development and fibrosis progression. Female gender, stage of liver fibrosis, degree of inflammation, especially rosette formation, predicted fibrosis progression and cirrhosis. No association was found between follow-up biopsy findings and patients' prognosis or probability of developing cirrhosis.

P1158

DOES A NORMAL IgG INDICATE HISTOLOGICAL REMISSION IN AUTOIMMUNE HEPATITIS (AIH)?

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Background and Aims: Response to immunosuppressive treatment in AIH is often monitored by measurement of serum immunoglobulin G (IgG) as well as ALT. It is commonly assumed that serum IgG level correlates with histological activity (or Ishak necroinflammatory score: NIS) on liver biopsy, the historical "gold standard". However, only one group (Luth et al. 2008; J Clin Gastro 42(8): 926–930) have examined this relationship, finding that normalisation of both serum ALT and IgG reliably predicted a NIS of <6 but not a NIS of <4 (corresponding to minimal hepatitis, seen in less than half of the patients presumed to be in remission). Here, we aimed to reassess how well serum IgG correlated with NIS in treated patients with AIH undergoing follow-up biopsy for confirmation of disease remission.
Methods: We assessed 34 follow-up biopsies, performed to confirm histological remission in 28 patients with AIH (International Group criteria; Alvarez J, Hepatol 1999; 31: 929) on immunosuppressive treatment and an accompanying serum IgG (measured within 6 weeks of biopsy).
Results: For 32 of the 34 follow-up biopsies, accompanying serum IgG was in the normal range (≤16 g/L). However, only 13 of these 34 (38%) biopsies showed NIS <4 (minimal hepatitis). On ROC analysis, area under the curve (AUC) for IgG in predicting a NIS of ≥4 (n = 34) was 0.557 (p = 0.583). Sensitivity and specificity of IgG (cut off >16 g/L) in predicting a NIS of ≥4 were 5% and 92% respectively. Corresponding positive (PPV) and negative (NPV) predictive values were only 50% and 38% respectively. AUC for change in IgG (ΔIgG: baseline values minus values accompanying follow up biopsy; n = 33) in predicting NIS ≥4 was 0.571 (p = 0.501). Defining histological remission instead as *minimal or mild* hepatitis – NIS <6 (as Luth's group did because they found that such patients did not develop fibrosis progression), there were still 4/34 patients (12%) with normal serum IgG who were not in histological remission. On ROC analysis, AUC for IgG in prediction of NIS ≥6 on follow-up biopsy (n = 34) was 0.655 (p = 0.274). PPV and NPV for serum IgG (cut off >16 g/L) in predicting NIS ≥6 were 50% and 88% respectively. AUC for ΔIgG in prediction of NIS ≥6 was 0.586 (p = 0.547).
Conclusions: In treated patients with AIH, normalisation of serum IgG is *not* reliably predicative of histological remission. Confirmation of remission still requires a follow-up liver biopsy.

P1159

IDENTIFICATION OF SERUM METABOLITES ASSOCIATED WITH CHOLESTATIC PRURITUS

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Background and Aims: The pathogenesis of pruritus, a common and disabling symptom in patients with chronic cholestasis, is unknown but recently itching has been associated with increased levels of lysophosphatidic acid and some bile acids. Moreover, earlier analysis selected a metabolomic profile of itching. Therefore, we have expanded our analyses by measuring the serum concentration of these potential metabolites to better identify those associated with pruritus.
Methods: Serum samples were obtained from 85 patients with primary biliary cirrhosis, 21 with pruritus and 64 without pruritus. All patients received ursodeoxycholic acid (13–16 mg/kg/d) for the duration of the disease which was similar in the two groups (8.3±1.4 years). Methanol serum extracts were analyzed using an ultra performance liquid chromatography-time-of-flight-MS based platform. Then, 45 lipids were quantified according to an appropriate internal standard, including fatty acyls, bile acids, and lysoglycerophospholipids.
Results: Most analytes were elevated in the patients with pruritus as compared with those without. Pamitoyl-L-carnitine, bile acids, particularly primary bile acids (tauro and glycochenodeoxycholic), fatty acids (linoleic, octadecatrienoic, arachidonic, eicosatetraenoic, docosapentaenoic, dodecanoic, myristoleic, 15-HETE-hydroxy-eicosatetraenoic), lysophosphatidyl-cholines (myristoyl, pentadecanoyl, heptadecanoyl, hexadecyl, 1-O-octadecyl), lysophosphoethanolamines (tetradecanoyl and hexadecanoyl), lysophosphoinositol (palmitoyl, and oleoyl), N-acyl ethanolamides (palmitoyl and oleoyl) and pregnenolone sulfate were significantly higher in patients with pruritus. The

multivariate analyses identified, however, taurochenodeoxycholic acid (2.85±0.76 *vs* 0.40±0.29 μM, p < 0.001), 1-myristoyl-2-hydroxy-sn-glycero-3-phosphocholine (2.38±0.31 *vs* 1.33±0.07 μM, p < 0.0001), and docosapentaenoic acid (1.15±0.46 *vs* 0.20±0.02 μM, p < 0.0001), as the analytes independently associated with pruritus.

Conclusions: Pruritus in patients with primary biliary cirrhosis is associated with marked changes in the circulating concentration of different analytes, thus identifying one bile acid, one lysophosphatidylcholine and a free fatty acid as the major potential triggers of itching of cholestasis.

P1160

ANTI-GP210 ANTIBODY IS A SEROLOGICAL MARKER FOR THE PROGRESSION TO END-STAGE ENLARGED LIVER WITH MICRONODULAR CIRRHOSIS IN PRIMARY BILIARY CIRRHOSIS

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Background and Aims: We previously reported that explanted livers from patients with primary biliary cirrhosis (PBC) at the time of liver transplantation (LT) were morphologically classified into non-cirrhotic, macronodular, mixed nodular, and micronodular cirrhosis. In addition, we previously reported that positive anti-gp210 antibodies is a strong risk factor for the progression to end-stage hepatic failure in Japanese patients with PBC. However, the association of cirrhotic pattern with anti-gp210 antibodies has not been determined.

Methods: A total of 47 PBC patients (female 45, male 2), in whom the sera before or at the time of LT (age 32–69, median 55 years) were available, were registered from June 2010 to July 2014. Anti-mitochondrial antibodies (AMA), anti-gp210 and anti-centromere antibodies were measured by ELISA. The biochemical and immunological data were compared among 4 different types of cirrhosis.

Results: AMA, anti-gp210 and anti-centromere antibodies were positive in 80.9%, 59.6% and 19.1% of the enrolled 47 patients, respectively. The explanted livers were classified into non-cirrhotic (n = 3), macronodular (n = 9), mixed nodular (n = 9), and micronodular (n = 26) cirrhosis. There were significant differences between micronodular and macronodular cirrhosis in age at liver transplantation (53.5±5.6 vs 59.0±6.1, p = 0.0242), ratio of explanted liver volume to standard liver volume (ELV/SLV) (1.28±0.49 vs 0.79±0.12, p = 0.0097), ALP (743.8±523.5 vs 302.5±73.0 IU/ml, p = 0.017), IgG (2392.0±825.5 vs 1694.7±590.1 mg/dl, p = 0.027) and the positivity of anti-gp210 antibodies (76.9% vs 22.2%, p = 0.0060). The values of these variables in mixed nodular cirrhosis were between those of micronodular and macronodular cirrhosis.

Conclusions: These results indicate that there are two different types of PBC progression to end-stage hepatic failure, enlarged liver with micronodular cirrhosis and atrophic liver with macronodular cirrhosis, and that the former type of progression is represented by positive anti-gp210 antibodies which is a risk factor for more severe bile duct loss, interface hepatitis, and poor biochemical response to treatment. The underlying mechanism for anti-gp210 negative-type progression to end-stage hepatic failure remains to be clarified.

P1161

ROLE OF PNPLA3 FOR CHOLESTATIC LIVER AND BILE DUCT INJURY IN MICE

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Background and Aims: PNPLA3 belongs to a novel group of nutritional controlled lipid metabolizing enzymes known as the patatin-like phospholipase domain-containing (PNPLA) family. PNPLA3 has triglyceride hydrolase and/or lysophosphatidic acid acyltransferase activities. One genetic variant of PNPLA3, the I148M isoform, is strongly associated with non-alcoholic fatty liver disease (NAFLD) and progression towards fibrosis and cirrhosis in humans. Additionally, recent data indicate that the I148M correlates with susceptibility to progression of cholangiopathies such as PSC. Therefore we endeavoured to test whether the absence of PNPLA3 mitigates or exaggerates cholestatic liver, bile duct injury and biliary fibrosis induced by bile duct ligation (BDL) or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) feeding as mouse models of sclerosing cholangitis.

Methods: PNPLA3 Knockout (KO) and control mice (WT) were fed either normal chow or 0.1% DDC chow for 12 days. Additionally a cohort of KO and WT mice were subjected to BDL for 7 days.

Results: PNPLA3 is expressed in mouse primary cholangiocytes at levels comparable to hepatocytes (Ct value 29.8 and 27.6 respectively). At baseline WT and KO mice had no changes in bile flow and composition. After BDL both WT and KO mice had similar increases in liver weight/body weight ratios compared to controls whereas DDC feeding induced no liver weight changes. Increases of serum ALT, AST, bile acids, bilirubin and alkaline phosphatase were observed in both WT and KO mice post DDC feeding and BDL, but the differences between the treated groups were not significant. Liver histology/immunohistochemistry illustrated DDC feeding and BDL caused increases of collagen deposition, inflammation and ductular proliferation but no differences were observable between genotypes. Furthermore, DDC feeding significantly induced Cyp2b10, Cyp3a11, OSTβ and MRP3 mRNA expression in WT and KO mice but to similar levels. Additionally, markers of fibrosis, inflammation and biliary damage (i.e. Col1a1, IL6, F4/80, VCAM1, CK19) were increased at both the mRNA and protein levels in response to cholestatic injuries (BDL, DDC) but again differences were not significant between WT and KO mice.

Conclusions: Lack of PNPLA3 in mice does not mitigate or exaggerate DDC feeding or BDL induced cholestasis, hepatic inflammation, ductular proliferation and biliary fibrosis indicating that PNPLA3 may not play a role in cholangiocytes during cholestatic liver and bile duct injury.

P1162

MULTI-CENTRE AUDIT OF MANAGEMENT AND OUTCOME IN AUTOIMMUNE HEPATITIS (AIH) – PRELIMINARY RESULTS

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Background and Aims: There are limited data on demographic and presenting features of AIH outside large centres. We are conducting an outcome audit of AIH in 29 UK centres of varying size. We report

demographic, clinical and laboratory features at presentation in this cohort.

Methods: We collected all prevalent (still-attending) cases since 2000 and all incident cases since 2007 by searching electronic patient letters, histology databases and hospital coding. Case validation was by 1999 IAIHG diagnostic criteria. Information was collected and inputted into a web-based data collection system.

Results: Of 957 patients (209 prevalent, 748 incident) with, or treated as AIH, 79% were women. Age at diagnosis was [median (range)] 56 yrs (8–86): 6% were <21 yrs, 17% 21–40 yrs, 39% 41–60 yrs, 36% 61–80 yrs and 2% >80 yrs. Age distribution was similar in women and men. There were 91% Caucasian, 7% Asian, 1% Afro-Caribbean, 0.1% Chinese and 0.3% other groups. 43% had a personal or family history of autoimmune disease, including 15% with thyroiditis/hypothyroidism, 11% PBC, 5% IBD and 3% coeliac disease. HBV and HCV serology was negative in 914 patients (96%) and was undocumented in 39 (4%). Four patients (0.4%) had acute hepatitis IgM antibodies (2 CMV, 1 EBV and CMV, 1 HEV). ANA was present in 49%, ASMA in 41% and Anti-LKM1 in 2%; at least one was present in 78%. AMA was found in 9%. Serum IgG/globulin was raised in 76%. Fifty patients (5%) did not meet IAIHG diagnostic criteria but were treated as AIH.

Presenting symptoms were: none 23%, jaundice/itching 41%, fatigue 32%, nausea, weight loss and abdominal pain each 14%, joint aches 12%, flu-like symptoms 7%, rash 3%, amenorrhoea 0.3%, others 5%. Time from first abnormal liver tests to diagnosis was 3 months (0–167) and was shorter in patients presenting with jaundice/pruritus than in the others: 1 (0–167) vs 5 (0–131) months ($p \leq 0.0001$). At presentation, 4% had ascites, 5% oedema, 1.5% encephalopathy, 0.6% variceal bleeding and 8% had clinical decompensation (defined as ≥1 of these). In an additional 13%, MELD score was >15. 96% of patients had a diagnostic liver biopsy. 21% had cirrhosis (Ishak fibrosis 5/6).

Conclusions: In this large multicentre cohort of AIH patients, 21% had cirrhosis and 21% had liver decompensation at presentation. Delay in diagnosis was often many months, especially in those without jaundice. 4% did not have documented HBV/HCV serology and 5% did not meet IAIHG criteria.

P1163
BASELINE LIVER BIOPSY IN AUTOIMMUNE HEPATITIS (AIH): CONTRIBUTION TO DIAGNOSIS AND PREDICTION OF TREATMENT RESPONSE

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Background and Aims: Although recommended in published guidelines, diagnostic liver biopsy is often not performed in patients with suspected AIH: only 62% of UK consultants do so 'routinely' (Dhaliwal Gut 2012:62). Likewise, the uses and the choice of International AIH Group diagnostic criteria (full 1999 or simplified 2008) are not established.

Aims: Assessment, in patients with AIH by 1999 criteria, of the utility of (a) liver histology in diagnosis and prediction of treatment response and (b) the 2008 (simplified) IAIHG diagnostic criteria.

Methods: 90 patients [71 women, median age (range) 61 (14–84) years], presenting since 2006 and undergoing diagnostic liver biopsy for suspected AIH. Histology was assessed (using the 1999 and 2008 criteria and the Ishak scoring system) by one Liver Histopathologist (AKD) without reference to other results.

Results: Interface hepatitis was found in 87 (97%) of biopsies, a mainly plasma-cell infiltrate in 74 (82%), rosettes in 68 (76%), biliary changes in 15% and steatosis in 26%. Prior to biopsy, patients had 11 (8–19) diagnostic points (1999 criteria) and 11 did not have the required 10 points. Biopsy contributed 5 (0–6) more points and all patients then met the 1999 criteria (61 definite, 29 probable). In 49 patients with ≥13 pre-biopsy points, biopsy [contributing 4 (0–5) more points] was not needed for AIH diagnosis but detected biliary changes in 10 patients (resulting in PBC overlap diagnosis and UDCA treatment in 3) and cirrhosis in 9. Histological features did not differ between patients with (n = 68) and without (n = 22) serum antibodies (ANA, ASMA, LKM), between those with (n = 72) and without (n = 10) raised serum IgG, or between treated patients who did (n = 75) and did not (n = 8) achieve normal serum ALT within 6 months. Seventeen of the 90 patients (19%) did not meet the 2008 (simplified) criteria for AIH. In these, necroinflammatory score was 9 (2–16), fibrosis score was 3 (1–6) and, of 14 treated patients, 13 achieved normal serum ALT within 6 months.

Conclusions: Liver biopsy is important for diagnosis of AIH in those with lower, but less so in those with higher, pre-biopsy IAIHG (1999) diagnostic scores. It may also detect cirrhosis or biliary disease. Histological features are not associated with immunological features or treatment response. The 2008 simplified diagnostic criteria will "miss" 19% of patients with AIH by 1999 criteria. Most of these patients have significant disease and respond to treatment.

P1164
IMPAIRED EXPRESSION OF ENZYMES RESPONSIBLE FOR BILE ACID SYNTHESIS AND DETOXIFICATION IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS

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Background and Aims: Nuclear receptors (NR) coordinate responses aimed at protecting hepatocytes from toxic bile acid (BA). In experimentally induced cholestasis the complex interplay of several pathways under control of FXR, PXR and CAR lead to reduction of BA synthesis and enhanced expression of enzymes responsible for phase I and phase II modification. Whether comparable phenomenon operates in humans requires to be elucidated. The aim of the study was to find out whether self-protective mechanisms against retaining BAs are induced in response to prolonged cholestatic injury in PSC.

Methods: Human tissue specimens were obtained from explanted livers of patients with PSC (n = 11) and primary biliary cirrhosis (PBC, n = 20). Non-cholestatic liver specimens (n = 20) served as controls. Liver protein expressions of FXR, PXR, CAR, SHP alongside with CYP7A1 (BA synthesis), CYP3A4 (phase I hydroxylation), and SULT2A1 (phase II conjugation) were assessed by Western Blot.

Results: The protein levels of FXR and its downstream target gene SHP were significantly enhanced in comparison to controls in both PSC (5.4 fold, $p < 0.0001$ and 2.9 fold, $p < 0.0001$, respectively), and PBC (2.4 fold, $p = 0.03$ and 2.8 fold, $p < 0.0001$, respectively). Hepatocellular expression of FXR-dependent fibroblast growth factor FGF19 and its receptor FGFR4 were highly induced in PSC (45.1 ± 9.1 vs. 1.1 ± 0.1, $p = 0.001$ and 2.6 ± 0.5 vs. 1.0 ± 0.1, $p = 0.03$, respectively). Despite these changes the suppression of a rate limiting enzyme for BA synthesis CYP7A1 was not seen in PSC whereas it was present in PBC (0.54 ± 0.1 vs. 0.96 ± 0.1; $p = 0.006$). PXR and CAR protein levels were increased both in PSC (2.7 fold; $p = 0.04$ and 2.4 fold; $p < 0.0001$, respectively) and PBC (5.9 fold, $p < 0.0001$ and 3.1 fold; $p < 0.0001$, respectively). The expression CYP3A4 enzyme was again not changed in PSC but enhanced in PBC (2.2 ± 0.5 vs. 1.1 ± 0.1, $p = 0.02$). Similarly, the level of SULT2a1 enzyme was unchanged in PSC but upregulated in PBC (2-fold, $p < 0.0001$).

Conclusions: Significant differences between expression of crucial enzymes responsible for bile acids metabolism and hepatoprotection exist between PSC and PBC. Potential impairment

in repression of bile acids synthesis and alterations in detoxification mechanisms in PSC may be accountable for bile acid induced liver injury observed in this cholestatic condition.
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P1165
HIGH SEROPREVALENCE OF HEPATITIS E IN PATIENTS WITH AUTOIMMUNE HEPATITIS

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Background and Aims: Incidence of autochthonous hepatitis E virus (HEV) infection is reported to increase in Europe and evolution of chronic hepatitis E has been shown in patients receiving immunosuppressive treatment after solid organ transplantation. HEV infection may mimic autoimmune hepatitis or disease flares during immunosuppressive treatment. Aim of the present study was to investigate the frequency of HEV infection in patients with AIH.
Methods: Sera from seventy-two consecutive patients with AIH (female: 55 [76.4%], age: 42.2±17.5 years [mean±SD]) in a tertiary referral center were retrospectively analyzed at baseline and during treatment. All patients were scored as probable or definite AIH according to the simplified and/or revised scoring system of the IAIHG; prevalence of anti-HEV-antibodies (Beijing Wantai Biological Pharmacy Enterprises Co., Ltd.) and HEV RNA was determined.
Results: 19 (26.4%) patients tested positive for HEV-IgG antibodies, which was more than twice as high as the previously reported sero-prevalence in healthy Austrian individuals (12.4%). No patient tested positive for HEV-RNA. Seropositive patients were older (51±12 vs. 39±18 years, [means±SD], $p < 0.01$) but did not differ significantly in AST levels (14.6 [1.3–121.5] vs. 8.6 [0.7–84.3], ×ULN, median [range]), ALT levels (20.1 [1.6–62.7] vs. 11 [0.8–75.7], ×ULN, median [range]), bilirubin (1.8 [0–32] vs. 1.9 [0–58], mg/dl, median [range]) and IgG-levels (1.3 [0.7–2.7] vs. 1.3 [0.6–3.8], ×ULN, median [range]). Seropositivity did not have an effect on treatment response after 6 or 12 months of immunosuppressive treatment, respectively.
Conclusions: Seroprevalence of HEV-IgG was found to be high in our cohort with AIH patients suggestive that HEV infection might be a possible trigger for development of AIH. Testing for HEV RNA is advisable in patients examined for AIH or not responding to immunosuppressive treatment.

P1166
DECREASED SERUM DNase1 ACTIVITY IN PATIENTS WITH AUTOIMMUNE LIVER DISEASES

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Background and Aims: Deoxyribonuclease 1 (DNase1) is an endonuclease involved in chromatin degradation of necrotic cells during programmed cell death, thus its deficiency results in accumulation of DNA from nuclear antigens and may promote susceptibility to immune disorders such as systemic lupus erythematosus and Sjogren's syndrome. The aim of the study was to assess the role of DNase1 serum activity in patients with autoimmune liver diseases as similar data is missing in these groups of patients.
Methods: DNase1 activity was determined by single radial enzyme-diffusion method in serum samples obtained at the time of diagnosis of 224 patients with autoimmune hepatitis (AIH), 249 patients with primary biliary cirrhosis (PBC) and 36 patients with primary sclerosing cholangitis (PSC). Serum samples from 146 patients with chronic viral hepatitis B and C, 140 patients with non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH) and 114 healthy served as the disease control groups and healthy control group, respectively. Serum samples from 50 AIH and 39 PBC patients at least one year after appropriate treatment initiation were also available for paired analyses.
Results: DNase1 activity was significantly lower in AIH, PBC and PSC patients compared either to chronic viral hepatitis ($p < 0.02$, $p < 0.001$, and $p = 0.03$, respectively) or NAFLD/NASH ($p < 0.001$, for each comparison) and healthy control group ($p < 0.001$, for each comparison). No significant difference was found in between each specific autoimmune liver disease. In AIH patients, complete responders to treatment characterized by increased baseline DNase1 serum levels compared to partial responders, responders with relapses and non-responders ($p < 0.05$), while DNase1 activity was significantly increased after achievement of clinical and biochemical remission ($p < 0.001$).
Conclusions: DNase1 serum activity is significantly decreased in autoimmune liver diseases patients, suggesting a potential implication in pathogenesis. In particular, DNase1 activity could be used as a new surrogate biomarker of predicting response to treatment in AIH though further investigation is needed.

P1167
SHARED DISEASE-SUSCEPTIBILITY GENES BETWEEN PRIMARY BILIARY CIRRHOSIS AND CROHN'S DISEASE IN THE JAPANESE POPULATION

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Background and Aims: We previously identified *TNFSF15* as the most significant susceptibility gene at non-*HLA* loci for both primary biliary cirrhosis (PBC) and Crohn's diseases (CD) in the Japanese population. To further identify shared disease-susceptibility genes between PBC and CD, the associations of PBC and CD were studied with susceptibility genes to CD and PBC, respectively, in the Japanese population.
Methods: We selected 15 and 34 genetic variants which were significantly associated with PBC and CD, respectively, with reference to previously reported genome wide association studies and replication studies in the Japanese population. Association study was independently performed for these genetic variants in CD cohort (1312 CD patients and 3331 healthy controls) and PBC cohort (1279 PBC patients and 1015 healthy controls) in the Japanese population.
Results: Two susceptibility genes to CD, *ICOSLG* (rs2838519, $p = 3.85 \times 10^{-2}$) and *IL12B* (rs6556412, $p = 8.40 \times 10^{-3}$), were significantly associated with PBC, and three susceptibility genes to PBC, *CXCR5* (rs6421571, $p = 2.72 \times 10^{-2}$), *STAT4* (rs7574865, $p = 4.04 \times 10^{-2}$), *NFKB1* (rs230534, $p = 2.16 \times 10^{-2}$), were significantly associated with CD. The risk alleles of *ICOSLG* rs2838519 and *CXCR5* rs6421571 were the same between the two diseases, whereas those of *IL12B* rs6556412, *STAT4* rs7574865, and *NFKB1* rs230534 were opposite.

Conclusions: In addition to *TNFSF15*, *ICOSLG* and *CXCR5* revealed to play an important role in the shared disease-pathways (*ICOSLG-CXCR5* axis) between PBC and CD in the Japanese population. On the other hand, *IL12B*, *STAT4*, *NFKB1* might be involved in oppositely directed manner in the two diseases, indicating the different regulation of Th1/Th17 balance between the two diseases.

P1168

PROGNOSTIC CLINICAL BIOMARKERS OF CHOLESTATIC LIVER INJURY: PERTURBATION OF BILE ACID METABOLISM AND REACTIVE OXIDATIVE STRESS MARKER

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Background and Aims: During the clinical diagnosis of liver injury, clinicians must make an early assessment of the extent of progression from cholestasis in the bile canaliculi to severe hepatocellular injury, and subsequently make an appropriate decision with regard to the therapeutic strategy. In particular, cholestasis is difficult to diagnose by conventional markers at an early stage of disease. The aim of this comprehensive study was to identify definitive biomarkers for distinguishing symptom-based types of liver injury (e.g., hepatocellular injury, cholestasis) using blood samples of patients with liver disease.
Methods: A total 304 blood samples from patients with liver disease (n = 150) and 46 blood samples from healthy volunteers were obtained. The associations between the serum levels with 29 markers of interest such as bile acid components, oxidative stress markers and liver fibrosis markers and the liver injury types were assessed by a multiple logistic regression analysis. Receiver operating characteristic (ROC) analyses were performed to determine the optimal cut-off values of the significant biomarkers for identifying liver injury types.
Results: Multiple logistic regression analysis revealed that reductions in the lithocholic acid (LCA) and deoxycholic acid (DCA) levels, and elevation of the serum sulfated bile acid (SSBA), liver fibrosis marker IV collagen (type IV collagen), hyaluronic acid (HA) and reactive oxygen species (ROS) levels were all significantly associated with cholestasis. On the other hand, elevations in the LCA and type IV collagen levels, and a reduction in the ursodeoxy cholic acid (UDCA) level, were significantly associated with hepatocellular injury. The ROC analyses showed that the largest area under the ROC curve (AUC) was found for ROS, followed by DCA, HA, LCA, SSBA and type IV collagen in the cholestatic-type cases.
Conclusions: The present results of prospective study indicated that reactive oxygen species, lithocholic acid, deoxycholic acid and the sulfated bile acids are promising biomarkers for cholestasis and for distinguishing the types of liver injuries. The proposed diagnostic approach using biomarkers will allow us to accurately diagnose liver injury and to select an appropriate personalized therapy at onset or in the early stage.

P1169

OUTCOME INDICATORS IN PRIMARY SCLEROSING CHOLANGITIS: INTERIM ANALYSIS OF THE VALUE-BASED MEDICINE IN HEPATOLOGY STUDY

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Background and Aims: Primary sclerosing cholangitis (PSC) is an enigmatic disease with scarce therapeutic options, potentially evolving to severe and life-threatening complications, including malignancies like cholangiocarcinoma (CCA) or colon cancer. The clinical management of PSC remains challenging and may benefit from identification of outcome indicators to assess the quality of care.
This study aims to: (A) identify Outcome Indicators (OIs) for PSC, and (B) validate OIs in a clinical context with a preliminary interim analysis.
Methods: (A) A panel of experts generated a list of OIs using a modified version of the Delphi method. (B) OIs with the highest RAND/UCLA score were tested in an ongoing multicentric and prospective study (Value-based Medicine in Hepatology, VBMH).
Results: Five OIs were identified on the basis of the highest rating values and disagreement indexes next to 0. They include: annual rate of acute cholangitis episodes (OI#1); mortality rate for patients not yet listed for liver transplantation (OI#2); rate of quality of life improvement, measured by EQ-VAS (a visual assessment scale ranging from 0 to 100 where the patient points out his present-day health status) and EQ-5D (assessment of 5 domains that measure daily performance according to 3 levels of severity) (OI#3); number of patients died for CCA and CRC (OI#4); incidence and/or worsening of osteoporosis, expressed as T-score differential over a 2-year interval (OI#5). In the validation study, 63 consecutive patients with PSC enrolled in 3 tertiary liver centres in Northern Italy were evaluated for a 24-month follow-up period. For each OI, the following values were reported: OI#1: cumulative incidence of 5.2%, resulting in 0.029 cholangitis/patient; OI#2, OI#4: no patients died without being listed for transplantation or because of cancer during study time; OI#3: 38.9% and 19.4% of patients showed an improvement in EQ-VAS and EQ-5D parameters, respectively; OI#5: 3% of patients developed or worsened osteoporosis.
Conclusions: Five OIs for PSC were identified on a highly shared consensus. Albeit the study population is small (as in the case of rare diseases) and the follow-up time is short as compared to the long natural history of the disease, these OIs have proven to be easy to collect and to work appropriately. Therefore, they are suitable to be extended to specialized centres involved in PSC management to further validate their clinical usefulness.

P1170

THE ROLE OF GROWTH HORMONE RECEPTOR IN LIVER FIBROSIS AND CANCER

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Background and Aims: Growth hormone (GH) resistance and low serum levels of insulin growth factor 1 (IGF-1) have been associated with liver cirrhosis in humans, suggesting an important role for growth hormone receptor (GHR) in this disease. GHR itself controls various cellular functions including the transcription of IGF-1 through signal transducer and activator of transcription 5 (Stat5) signaling. In order to elucidate whether the growth hormone receptor (GHR) plays an active role in the establishment of fibrosis liver diseases or rather happens to be a major consequence of this illness, we crossed mice lacking the *Ghr/bp* gene ($Ghr^{-/-}$) with a mouse model of inflammatory cholestasis and liver fibrosis, the Mdr2 Knockout mouse ($Mdr2^{-/-}$).
Methods: Serum parameters and bile acid contents were analysed. Additionally histological stainings, western blotting and RT-PCRs were conducted to gain mechanistic insights.
Results: Our results indicate that $Ghr^{-/-}$;$Mdr2^{-/-}$ mice show deregulation of bile acid homeostasis and increased serum markers associated with inflammation and fibrosis. Bile duct proliferation and extensive collagen deposition were also observed in $Ghr^{-/-}$;$Mdr2^{-/-}$ compared to $Mdr2^{-/-}$ mice, suggesting that $Ghr^{-/-}$;$Mdr2^{-/-}$ developed a severe liver fibrosis phenotype. Additionally, a greater down regulation of the hepato-protective genes *Hnf6*, *Egfr* and *Igf1* accompanied by increased apoptosis was seen in $Ghr^{-/-}$; $Mdr2^{-/-}$ compared to control mice. Moreover, single knockout mice ($Ghr^{-/-}$) developed bile infarcts when fed with 1% cholic acid compared to Wt controls, indicating that hepatocytes upon loss of GHR become more susceptible to toxic bile acid accumulation. Surprisingly, $Ghr^{-/-}$;$Mdr2^{-/-}$ mice showed a significant decrease in tumor incidence compared to $Mdr2^{-/-}$ mice despite their severe fibrotic phenotype indicating that loss of GHR signaling may slow the progression from fibrosis/cirrhosis to cancer in the liver.
Conclusions: These findings suggest that loss of GHR signaling severely increase liver fibrosis in a mouse model of inflammatory cholestasis, signifying the possible therapeutic value of this pathway in the development of liver fibrosis treatments.

P1171

EPIDEMIOLOGY AND DISEASE BURDEN OF PRIMARY BILIARY CIRRHOSIS IN SOUTH KOREA: A NATIONWIDE, POPULATION-BASED STUDY

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Background and Aims: The epidemiology of primary biliary cirrhosis (PBC) has wide geographic variation. There is no data on the prevalence and incidence of PBC in South Korea. The aim of this study was to elucidate the population based nationwide prevalence and incidence of PBC in South Korea.
Methods: We used the data from nationwide population-based Health Insurance Review and Assessment Service claims database and Rare Intractable Disease registration database, which includes physician-certified diagnoses based on uniform criteria for primary biliary cirrhosis from 2008 to 2013. Age-specific incidence and prevalence were calculated, and data on comorbidity and medical costs were also retrieved.
Results: The total number of patients with PBC in South Korea was 2347 in 2013; the prevalence rate in 2013 was 4.61 per 100,000 population, 8.06 in female and 1.16 in male. The incidence rates from 2011 to 2013 were 0.84, 0.92 and 0.87 per 100,000 population, respectively. Female was 87.4% of patients and peak age of new cases was 6th decades. Common comorbidities were hyperlipidemia (17%), hypothyroidism (4.5%) and various extrahepatic autoimmune diseases (2.1%). Common complications were ascites (9.9%), variceal bleeding (4.1%), and hepatocellular carcinoma (1.3%). A total of 71 patients (2.5%) had liver transplantation during the 5 years. The nationwide total direct medical cost was 8.5 million US dollars (USD) per year and the average cost for each patient was 950 USD in 2013.
Conclusions: This is the first report on the nationwide epidemiology of PBC in South Korea. We found the prevalence and incidence of PBC in Korea were lower than those of Western countries, while similar with those in Japan. Further study on the genetic or environmental factors related to the epidemiologic findings are warranted.

P1172

COLECTOMY IS ASSOCIATED WITH DEVELOPMENT OF CHOLANGIOCARCINOMA IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS AND INFLAMMATORY BOWEL DISEASE

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Background and Aims: Inflammatory Bowel Disease (IBD) occurs in two-thirds of patients with Primary Sclerosing Cholangitis (PSC) and cholangiocarcinoma (CCA) is estimated to have a lifetime incidence of up to 10% in patients with PSC. Despite these associations, the impact of colectomy in patients with PSC-IBD remains ill defined.
Aim: To identify the association between PSC-IBD and the occurrence of CCA in patients with and without colectomy.
Methods: Adult patients with PSC-IBD at the Mayo Clinic, Rochester from Jan 2005-Dec 2013 were identified. Medical records were retrospectively reviewed for relevant variables including gender, dates of individual diagnoses, occurrence and indication for colectomy, presence of decompensated liver disease, and development of outcomes including CCA, death, and liver transplant. CCA was defined by histologic evidence of malignancy

(biopsy or cytology) or consistent radiographic features. The primary analysis focused on time to development of CCA among those with and without colectomy. Overall rates of CCA within certain age and follow-up periods were determined. Cox proportional hazard models where colectomy was treated as a time-dependent covariate were used to determine the risk of CCA among patients with and without colectomy.

Results: The study cohort consisted of 404 patients with PSC-IBD. The mean age at diagnosis of IBD, PSC, and CCA was 29.4, 40.3 and 56.9 years, respectively. 115 (28%) subjects had a colectomy; 53 (46%) for colonic neoplasia and 38 (33%) for medically refractory disease. Overall the risk of CCA was increased in patients with prior colectomy (HR = 1.8, 95% CI 1.3–2.6, p = 0.002). The risk of CCA increased 40% for every 10 year increase in age (HR = 1.4, 95% CI 1.2–1.6). There was no significant evidence of an increased risk of CCA among those with refractory disease (HR = 0.87 95% CI 0.42–1.83, p = 0.72) or colonic neoplasia (HR = 1.26 95% CI 0.75–2.03) compared to those without colectomy. The rate of CCA per 1000 patient-years in patients 0–29 yrs, 30–39 yrs, 40–49 yrs, 50–64 yrs, and 65+ yrs that did not have a colectomy was 5.9, 16.1, 29.8, 37.7, and 31.9, respectively, compared to 44.0, 39.8, 42.6, 52.4, and 81.6 among those that did have a colectomy.

Conclusions: In this large cohort of patients with PSC-IBD, colectomy was associated with an almost two-fold increased risk of CCA. This association may assist in identifying a subset of patients who are at high risk of this dreadful complication.

P1173

MAIT CELLS ARE ENRICHED IN PORTAL TRACTS AND RESPOND TO BILIARY EPITHELIAL CELLS PRESENTING BACTERIAL LIGANDS DURING LIVER INFLAMMATION

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Background and Aims: Mucosal-Associated Invariant T (MAIT) cells are unconventional T-cells characterised by the invariant T-cell antigen receptor α-chain, Vα7.2, high expression of CD161 and restriction by the MHC class 1-related molecule, MR1. They are important for antibacterial immunity at mucosal sites; however, their role in the pathogenesis of hepatic inflammation remains unclear. Here we investigated the frequency, phenotype, localisation and function of liver infiltrating (LI) MAIT cells.

Methods: Blood and LI MAIT cells were phenotyped by flow cytometry. Intrahepatic localisation was examined by immunohistochemistry. MAIT cell activation in response to *E. coli*-exposed biliary epithelial cells (BEC) was assessed in a co-culture study ± anti-MR1.

Results: MAIT cells were enriched in liver vs. blood in normal (9% vs. 3% of T-cells, p < 0.01) and diseased (4% vs. 0.3%, p < 0.01) tissues. However, frequencies were lower in diseased vs. normal livers (4% vs. 9%, p < 0.01). In blood and normal livers most were $CD8^+$; however, the $CD4^+$ subset was increased in diseased livers (11% vs. 3% of $CD3^+$MAIT cells, p < 0.05). LI MAIT cells had a $CD45RA^-CCR7^-$ (88%) $CD69^+$ (80%) phenotype and expressed the serine exopeptidase CD26 (91%) as well as adenosine pathway markers CD39 (45%) and CD73 (21%). Human intrahepatic Vα7.2 cells resided in hepatic sinusoids and around bile ducts. Consistent with this distribution they expressed the sinusoid recruitment receptor CXCR3 (80%), integrins LFA-1 (91%) and VLA-4 (58%) and biliary homing chemokine receptors CCR6 (53%), CXCR6 (21%), CCR10 (4%) and integrin αEβ7 (23%). Gut homing integrin α4β7 (20%) and cytokine receptors IL-18Rα (84%) and IL23R (20%) were also detected. MAIT cells from diseased livers possessed mixed lineage phenotypes expressing RORc and Tbet and cytokines IL17, IL22, IFNγ and TNFα. They also produced cytolytic granzyme B. In response to *E. coli*-exposed BEC, MAIT cells expressed IFNγ and CD40L. These responses were inhibited by blocking with anti-MR1.

Conclusions: We provide first evidence that activated effector memory $CD3^+CD161^{++}Va7.2^+$ MAIT cells are enriched in human livers with an altered CD4/CD8 profile in disease. High expression of CXCR3/LFA-1/VLA-4 indicates recruitment via hepatic sinusoids and CCR6/CXCR6/αEβ7 suggest homing to the peri-biliary region where LI MAIT cells are activated in response to bacteria-exposed BEC in an MR1 dependent manner, leading to liver injury through their release of cytolytic enzymes and inflammatory cytokines.

P1174

MICROBIOTA-DEPENDENT MARKER TRIMETHYLAMINE-N-OXIDE (TMAO) IS ASSOCIATED WITH THE SEVERITY OF PRIMARY SCLEROSING CHOLANGITIS

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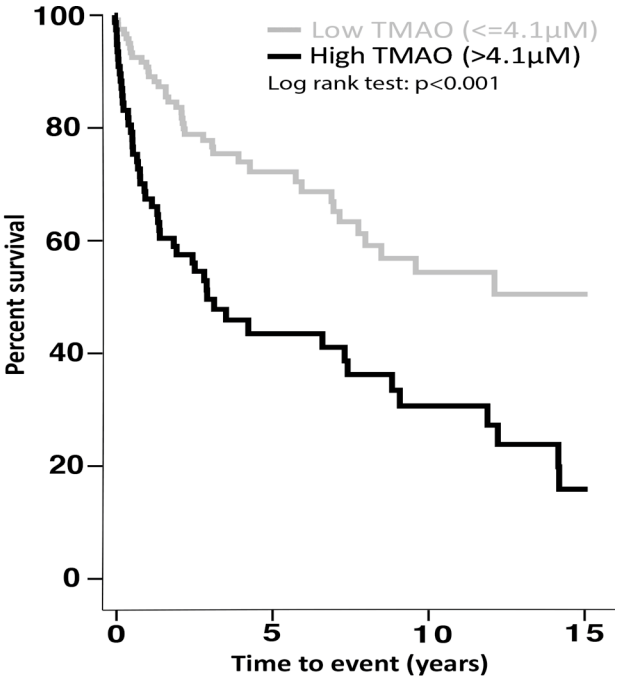
Background and Aims: Trimethylamine-N-oxide (TMAO) is produced in the liver from trimethylamine, which is exclusively generated by gut bacteria from dietary choline and carnitine found in e.g. red meat and dairy products. TMAO influences bile acid levels, metabolism and potentially also inflammation. Given the production in the liver, link to the gut microbiota and bile acid homeostasis, we aimed to investigate the regulation of TMAO in primary sclerosing cholangitis (PSC).

Methods: We measured serum TMAO in 305 PSC patients, 90 ulcerative colitis (UC) patients and 99 healthy controls (HC), with a median age (male %) of 41 (76), 38 (51) and 40 (60), respectively. Reduced liver function was defined by increased prothrombin time (International Normalized Ratio >1.2 or Normotest <70).

Results: TMAO level was lower in PSC patients than in UC and HC (medians 3.1, 4.0 and 3.5, respectively, p < 0.05 versus HC and UC). We hypothesized that this could be explained by reduced liver function affecting TMAO production. In line with this, TMAO in PSC with reduced liver function, but not TMAO in those with normal liver function, was lower than in HC (p < 0.05). The TMAO level in UC was higher than in PSC irrespective of liver function (p < 0.05), while TMAO was similar in PSC patients with and without inflammatory bowel disease. Focusing on PSC patients with normal liver function, the TMAO level was higher in patients reaching an end-point during follow-up (liver transplantation, n = 70, or all-cause death, n = 21) than those without (median 4.6 versus 2.7, p = 0.001). AUROC-analysis yielded an AUC of 0.64 (p < 0.001) with a suggested optimal TMAO cut-off of 4.1μM. When stratified by this value, PSC patients with high TMAO exhibited shorter transplantation-free survival than patients with low TMAO (median 3.0 versus 16.2 years, p < 0.001, Figure 1). In Cox regressions, TMAO

>4.1μM and Mayo risk score were independently associated with transplantation-free survival, with HR 2.0 (95% CI 1.2–3.1, p = 0.005) and 1.5 (95% CI 1.2–1.8, p < 0.001), respectively.
Conclusions: PSC patients with impaired liver function had decreased TMAO level, suggesting that TMAO may be influenced by reduced liver function. On the other hand, in PSC patients with normal liver function, elevated TMAO was associated with a shorter liver transplantation-free survival, suggesting that dietary factors and gut microbiota profile may be relevant for the disease prognosis in PSC.



	0	3	5	10	15
Number at risk					
Low TMAO	120	70	41	19	5
High TMAO	77	29	17	10	3

Figure 1.

P1175
ABILITY OF A SIMPLE RADIOLOGIC SCORE, ASSESSED BY THREE-DIMENSIONAL MAGNETIC RESONANCE CHOLANGIOGRAPHY (MRC), TO PREDICT CLINICAL OUTCOME OF PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS (PSC)

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Background and Aims: The natural course of PSC is highly variable and the use of current prognostic models is not recommended in an individual patient. The prognostic value of cholangiographic features assessed by endoscopic retrograde cholangiography has been suggested but cholangiography by magnetic resonance imaging (MRI) has become the radiologic standard for diagnosis of PSC. We have previously shown that 2 MRI risk scores can predict radiologic progression in PSC patients. The aim of this study was to evaluate the ability of these scores to predict clinical outcome of PSC patients.
Methods: This retrospective single center study included PSC patients who had not been studied in our previous work. Time of inclusion was defined by the date of the first three-dimensional MRC. Primary endpoint was transplantation free survival. Predictive value of clinical, biological and radiologic features [including the 2 MRI scores: MRI score without gadolinium = 1·(dilatation of intrahepatic bile ducts) + 2·(dysmorphy) + 1·(portal hypertension); MRI score with gadolinium = 1·(dysmorphy) + 1·(parenchymal enhancement heterogeneity)] were analyzed by Cox regression models.
Results: 67 patients were included. Their characteristics at baseline were (median [extremes]): age 36.8 years [14.2–76], male 63%, IBD 67%, ursodesoxycholic acid treatment 82%. Gadolinium administration was performed in 61% of patients. Median duration of follow-up was 3.62 years. 8 patients underwent liver transplantation and 2 patients died. In univariate analysis, factors associated with transplantation free survival were: bilirubin, ASAT, albumin and MRI score without gadolinium. Prognostic factors identified by multivariate analysis adjusted on age at entry were ASAT and MRI score without gadolinium. The performance of the MRI score without gadolinium for predicting clinical outcome was assessed by ROC analysis showing an AUC of 0.818 [0.691–0.946] ($p < 10^{-6}$). With a threshold at 2, this score had a sensitivity of 90% and a specificity of 56%.
Conclusions: A MRI risk score, that we have previously identified as associated with radiologic progression, appears also able to predict clinical outcome in PSC patients. Further steps include study of larger populations as well as external validation.

P1176
SERUM AUTOTAXIN IS ASSOCIATED WITH SIGNIFICANT IMPAIRMENT OF HEALTH RELATED QUALITY OF LIFE, CLINICAL AND BIOCHEMICAL FACTORS IN PRIMARY BILIARY CIRRHOSIS

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Background and Aims: Autotaxin (ATX) is involved in the synthesis of lysophoshatidic acid (LPA), a robust neuronal activator. Both parameters were recently linked to pruritus in cholestasis (Hepatology 2012;56:1391–1400), and it was hypothesized that ATX inhibitors could be useful for the treatment of cholestatic itch. The potential association between ATX and other relevant factors related to chronic cholestatic conditions has not been studied yet.
Methods: This study involved a group of 116 well characterized patients with PBC (M/F-9/107; mean age 58±12 years). PBC-40, PBC-27 and SF-36 questionnaires were used in the assessment of health related quality of life (HRQoL). Fifty-two patients had liver cirrhosis on histology or imaging. Total fasting serum bile salts (BS) and 27 BS metabolites were analyzed in 47 patients. ATX activity was quantified enzymatically.
Results: Serum ATX activity showed a significant correlation with pruritus assessed with the itch-specific domains of PBC-40 and PBC-27 questionnaires (r = 0.305; p = 0.001). It also correlated with serum AST (r = 0.234; p = 0.02) and ALP activities (r = 0.323; p = 0.0007). ATX was significantly higher in patients with cirrhosis (11.6±3.5 vs 9.3±4.7; p = 0.004) and correlated with the Mayo risk score (r = 0.451; p = 0.004). It also correlated with total fasting serum BS concentrations (r = 0.493; p = 0.0005). In multivariate analysis, glycine conjugates of lithocholate (LCA-Gly) and cholate (CA-Gly)

were independent variables related to ATX activity. In terms of HRQoL, serum ATX was associated with *fatigue* (r = 0.218; p = 0.02) in PBC-40 as well as *fatigue* (r = 0.217; p = 0.02), *cognitive* (r = 0.207; p = 0.03) and *emotional* (r = 0.202; p = 0.03) domains of the PBC-27 questionnaire. No correlations were found with generic SF-36 domains, except for *physical functioning* (r = 0.204; p = 0.03).

Conclusions: In patients with PBC, serum ATX is not only associated with pruritus but may also be involved in impairment of further aspects of patients' quality of life and liver dysfunction. Thus, ATX inhibitors could be of potential benefit not only in the treatment of pruritus but also other incapacitating symptoms related to chronic cholestasis.

P1177

RISK FACTORS FOR HEPATIC DECOMPENSATION IN PRIMARY BILIARY CIRRHOSIS – RESULTS OF AN INTERNATIONAL FOLLOW UP STUDY OF 2326 PATIENTS

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Background and Aims: Hepatic decompensation is an important complication in primary biliary cirrhosis (PBC). However, it remains unclear which factors best predict decompensation. Therefore we aimed to identify baseline factors predicting decompensation to support risk stratification purposes.

Methods: Long-term follow-up (FU) data of ursodeoxycholic acid (UDCA) treated patients was derived from 11 North American and European centers. Decompensation was defined as a first event of ascites, variceal bleeding, or encephalopathy. Patients with decompensation prior to baseline or within the first year of FU were excluded. Risk factor analysis was performed using Cox proportional hazard models. Biochemical non-response was defined by the Paris I criteria. Survival was defined as liver transplantation free survival.

Results: The study population consisted of 2326 UDCA treated PBC patients. 413 patients (18%) were excluded because of missing data, and 78 (3%) because of decompensation before baseline or within first year of FU. Of 1835 included patients (median FU 9.1 years, IQR 5.0–14.3), 163 developed decompensation [ascites: 70 (42.9%), variceal bleeding: 25 (15.3%), encephalopathy: 11 (6.7%), multiple events: 57 (35%)]. On multivariable analysis, the following baseline factors were independently predictive of occurrence of decompensation (Table): year of diagnosis (p = 0.007), moderate disease stage (abnormal bilirubin and/or albumin) (p < 0.001), advanced disease stage (abnormal bilirubin and albumin) (p = 0.013), higher serum alkaline phosphatase levels (p < 0.001), lower platelet count (p < 0.001), higher AST/ALT ratio (p < 0.001) and biochemical non-response after one year (p < 0.001). Among biochemical non-responders 3-, 5-, and 10-year decompensation rates were 8.2%, 15.1% and 24.8%, versus 0.7%, 1.3%, and 3.8% in biochemical responders. One-year survival of decompensated vs. non-decompensated patients was 65.6% vs. 99.7% respectively and 5-year survival was 26.2% vs. 93.6% (time dependent HR 33.9; 95% CI 22.3–51.7). Survival after decompensation did not significantly differ between ascites, variceal bleeding and encephalopathy.

Table: Univariable and multivariable analysis on associations with decompensation

	Univariable analysis		Multivariable analysis	
	HR	95% CI	HR	95% CI
Baseline				
Male gender	1.38	0.86–2.23		
AMA	0.95	0.58–1.57		
Age at start FU	1.01	0.99–1.02		
Year of diagnosis	0.93	0.92–0.95	0.97	0.95–0.99
Moderate disease stage [a]	5.29	3.82–7.34	2.40	1.68–3.42
Advanced disease stage [b]	5.61	3.14–10.03	2.22	1.19–4.16
Albumin	0.15	0.05–0.42		
Platelets (per 10 units)	0.91	0.90–0.93	0.93	0.91–0.95
LN bilirubin	3.55	3.00–4.20		
LN alkaline phosphatase	2.39	1.90–3.01	1.68	1.29–2.18
LN AST	2.68	2.11–3.42		
LN ALT	1.58	1.26–1.97		
LN AST/ALT ratio	2.24	1.63–3.07	2.23	1.55–3.21
LN APRI [c]	2.85	2.41–3.36		
After one year				
Biochemical non-response [d]	6.12	4.42–8.47	2.97	2.04–4.33

[a] Abnormal albumin or bilirubin; [b] Abnormal albumin and bilirubin; [c] AST/platelets ratio index; [d] Paris I Criteria.

Conclusions: Earlier year of diagnosis, advancing disease stage, higher alkaline phosphatase, lower platelet count, higher AST/ALT ratio, and biochemical nonresponse are independently associated with hepatic decompensation in PBC. Since survival following decompensation is poor, these factors might be useful for risk stratification.

P1178

MICRORNAS IN SERUM AND BILE OF PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS AND/OR CHOLANGIOCARCINOMA

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Background and Aims: Patients with primary sclerosing cholangitis (PSC) are at high risk for the development of cholangiocarcinoma (CC). Analysis of micro ribonucleic acid (MiRNA) patterns is an evolving research field in biliary pathophysiology with potential value in diagnosis and therapy. Our aim was to evaluate miRNA patterns in serum and bile of patients with PSC and/or CC.

Methods: Serum and bile from consecutive patients with PSC [n = 40 (serum), n = 52 (bile)], CC [n = 31 (serum), n = 19 (bile)] and patients with CC complicating PSC (PSC/CC) [n = 12 (bile)] were analyzed in a cross-sectional study between 2009 and 2012. The miRNA levels in serum and bile were determined with global miRNA profiling and subsequent miRNA-specific polymerase chain reaction-mediated validation.

Results: Serum analysis revealed significant differences for miR-1281 (p = 0.001), miR-126 (p = 0.001), miR-26a (p = 0.001), miR-30b (p = 0.001) and miR-122 (p = 0.034) between patients with PSC and patients with CC. MiR-412 (p = 0.001), miR-640 (p = 0.001), miR-1537 (p = 0.003) and miR-3189 (p = 0.001) were significantly different between patients with PSC and PSC/CC in bile.
Conclusions: Patients with PSC and/or CC have distinct miRNA profiles in serum and bile. Furthermore, miRNAs are differentially expressed in bile of patients with CC on top of PSC indicating the potential diagnostic value of these miRNAs.

P1179
CONNECTING THE LIVER AND GUT IN PSC: INTESTINAL CCL25 IS INCREASED IN COLITIS, CORRELATES WITH INFLAMMATORY ACTIVITY AND FACILITATES EFFECTOR CCR9+ LYMPHOCYTE RECRUITMENT

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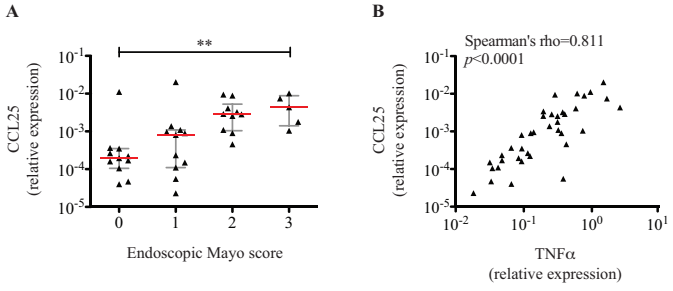
Background and Aims: Recruitment of mucosal T-cells to the liver in response to aberrantly expressed homing signals drives hepatobiliary inflammation in primary sclerosing cholangitis (PSC). This is exemplified by the 'gut-homing' chemokine CCL25, which recruits intestinal CCR9$^+$ effector cells to the PSC liver. However, previous studies report CCL25 expression as being confined to the small bowel whereas PSC is typically associated with colonic inflammation. Our aim was to determine whether CCL25 and CCR9 are expressed in the inflamed human colon in patients with ulcerative colitis.
Methods: Mucosal biopsies were obtained during surveillance colonoscopy (n = 40) and colonic tissue from patients undergoing surgical resection for refractory colitis (n = 6), or non-colitis-associated cancer (n = 5). CCL25 mRNA (relative to GUSB) was evaluated by qRT-PCR and protein expression confirmed using western blotting (relative to β-actin) and tissue-ELISA. Lymphocyte CCR9 expression was quantified by flow cytometry, and the ability of sorted $\alpha4\beta7^+CCR9^+$ and $\alpha4\beta7^+CCR9^-$ T-cells to transmigrate across hepatic sinusoidal endothelium (HSEC) investigated in flow-based adhesion assays.
Results: CCL25 mRNA and protein were absent from normal colon but present in ulcerative colitis. CCL25 mRNA expression correlated with endoscopic Mayo score (0.0029 vs. 0.00023; p = 0.0001) and tissue levels of TNFα (Spearman's rho 0.811; p < 0.0001) as indices of inflammatory activity (Figure 1). In colitis, 93% of CD4$^+$ and 16% of CD8$^+$ T-cells expressed CCR9 (6% and 2% in normal colon; p = 0.01 and 0.03) and were predominantly CD127$^+$ effector lymphocytes. $\alpha4\beta7^+CCR9^+$ T-cells showed enhanced transendothelial migration across HSEC compared with $\alpha4\beta7^+CCR9^-$ T-cells (60 vs. 30 cells/10^6 perfused; p = 0.045).



Conclusions: CCL25 is expressed in the inflamed human colon, correlates with inflammatory activity and is associated with high frequencies of CCR9$^+$ tissue-infiltrating lymphocytes. These findings lend further support to the mucosal lymphocyte homing hypothesis of PSC and show how it can be applied to colitis.

P1180
IDENTIFICATION OF PBC PATIENTS IN NEED OF ADDITIONAL THERAPY DURING THE COURSE OF UDCA TREATMENT – AN INTERNATIONAL MULTICENTER STUDY

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Background and Aims: In primary biliary cirrhosis (PBC) several biochemical criteria have been proposed to evaluate response to ursodeoxycholic acid (UDCA) after 1 year of therapy. All criteria are predictive of transplant-free survival, but with varying performance. We aimed to develop a prediction model identifying patients in need of additional therapy after 1 year and also during the course of therapy.
Methods: Data from 4119 UDCA-treated patients of the international Global PBC Study Group platform were included. The database was randomly split in a derivation (n = 2488, 60%) and validation cohort (n = 1631, 40%). Cox proportional hazard regression model was used to develop a model predicting transplantation-free survival based on a combination of biochemical and clinical variables after 1 year of UDCA therapy. Usefulness was tested up to 5 years follow-up. Predictive ability was tested with AIC and C statistic and compared with existing criteria. Finally, patients were sex-, age- and calendar time matched with the general Dutch population to identify patients with a significantly worse survival than the general population; hence most in need of additional therapy.

Results: In the derivation set 558 patients underwent liver transplantation or died during a median follow-up of 7.8 years and in the validation set 328 patients during a follow-up of 7.5 years. Variables included in our final model were: age at entry (HR 1.05, P < 0.0001), bilirubin (HR 2.56, P < 0.0001), albumin (HR 0.10, P < 0.0001), alkaline phosphatase (HR 1.40, P = 0.0002) and platelet count (HR 0.97, P < 0.0001) and a prognostic index was calculated based on the beta coefficients. The model at 1 year follow-up had a good performance in both the derivation (C statistic 0.81, 95% CI 0.79–0.83) and validation cohort (0.82, 0.79–0.84) and was better compared with Barcelona (0.58, 0.55–0.60), Paris-1 (0.69, 0.66–0.71), Rotterdam (0.69, 0.66–0.71), Toronto (0.61, 0.58–0.63) and Paris-2 criteria (0.63, 0.61–0.65). The model had a comparable performance after 2, 3, 4 and 5 years of UDCA therapy and did not lose predictive power. 50% of patients were identified with a survival significantly deviating from that of a matched population.
Conclusions: We showed and validated that during UDCA therapy liver transplant-free survival can accurately be predicted with a model comprising age, bilirubin, albumin, alkaline phosphatase and platelet count. The model is useful up to 5 years UDCA therapy. 50% of patients could potentially benefit of additional therapies to UDCA.

P1181
MICROBIOTAL PRODUCT ACTIVATION OF NLRP3 INDUCES IL-18 SYNTHESIS AND IMPAIRS THE EPITHELIAL BARRIER FUNCTION IN REACTIVE CHOLANGIOCYTES

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Background and Aims: Microbiobial products are hypothesized to be able to affect cholangiocyte biological response to injury and to participate to the development of cholangiopathies, in particular of Primary Sclerosing Cholangitis (PSC). Microbiotal products activate a signalling cascade defined as inflammasome, being NLRP3 one of the key molecules. NLRP3 induces IL-1β and IL-18 synthesis and release, thus modulating cell biology and eliciting pro-inflammatory signals. We aimed to verify whether NLRP3 inflammasome is activated in sclerosing cholangitis and which are its effects in reactive cholangiocytes.
Methods: NLRP3 expression was tested in cholangiocytes from normal and cholestatic livers. NLRP3 was knocked down *in vitro* by siRNA. *In vivo*, wild type (WT) and *Nlrp3*^{A350VneoR} (*Nlrp3*$^{-/-}$) mice were fed with 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC, a model of sclerosing cholangitis) for 4 weeks.
Results: NLRP3 is overexpressed in cholangiocytes of mice subjected to DDC and of patients affected by PSC, but not in normal conditions. *In vitro*, exposure of cultured cholangiocytes to LPS/ATP induced NLRP3 and interleukin (IL)-18 expression but not IL-6 or IL-1β expression and release. LPS/ATP also significantly decreased Zonluin-1 expression and did not affect cell proliferation. Knock down of NLRP3 expression by siRNA neutralized the effects of LPS/ATP on IL-18 synthesis and re-established Zonulin-1 expression. *In vivo*, the increases in the liver CK-19-positive parenchyma induced by 4 week DDC in WT animals were reduced in *Nlrp3*$^{-/-}$ mice.
Conclusions: NLRP3 is expressed in reactive cholangiocytes and its activation leads to IL-18 synthesis, Zonulin-1 downregulation and to impaired biological response to injury. These findings suggest that microbiotal products may participate to the development of certain cholangiopathies by activating the inflammasome in cholangiocytes and impairing the barrier function of the biliary epithelium.

P1182
MIR-506 IS UPREGULATED BY PRO-INFLAMMATORY CYTOKINES IN HUMAN CHOLANGIOCYTES AND INHIBITS CELL PROLIFERATION AND ADHESION

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Background and Aims: Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease associated with autoimmune phenomena targeting the intrahepatic bile duct cells (ie. cholangiocytes). Changes in the expression of miRNAs (miRs) were reported in livers from PBC patients compared to normal controls. We recently reported that the overexpression of miR-506 in PBC livers is preferentially located in the bile duct cells, where it promotes cholestasis through the direct targeting of both the Cl^-/HCO_3^- anion exchanger 2 (AE2) [Banales JM, *et al. Hepatology* 2012; 56(2): 687–697] and the inositol 1,4,5-trisphosphate receptor ($InsP_3R3$) [Ananthanarayanan M, *et al. J Biol Chem.* 2014; *in press*]. Here, we investigated the mechanisms of miR-506 overexpression and its direct role in the regulation of cholangiocyte pathophysiology.
Methods: Different sizes of miR-506 promoter (3000, 2000 or 1000 bp) were cloned in a luciferase expression vector, which were transfected in human cholangiocytes (H69 cells). The role of pro-inflammatory cytokines, bile acids, estrogens and glucocorticoids was evaluated on the promoter activities. MiR-506 or a negative controls miRNA sequence were also cloned in an expression vector under the regulation of the CMV promoter; these constructs were stably transfected in H69 human cholangiocytes, and the cell proliferation and adhesion were evaluated. Proteomic analysis of H69, H69-CMV-miR(−) and H69-CMV-miR506 were carried out.
Results: The 3000 bp miR-506 promoter (Z1) showed higher basal luciferase activity compared to the 2000 (Z2) and 1000-bp (Z3) constructs in H69 human cholangiocytes. The presence of pro-inflammatory cytokines found overexpressed PBC livers (ie, IL8, IL12, IL17 and IL18) upregulated the Z1 promoter activity but not the Z2 and Z3 activities. On the other hand, bile acids, estrogens and glucocorticoids did not alter the miR-506 promoter activities. Overexpression of miR-506 decreased cholangiocyte proliferation and adhesion. These functional alterations were linked to changes in the expression of proteins involved in the mitochondrial energetic metabolism and the cytoskeleton.
Conclusions: Upregulation of miR-506 in PBC cholangiocytes may be promoted by pro-inflammatory cytokines. MiR-506 inhibits the proliferation and adhesion of human cholangiocytes by regulating different metabolic and cytoskeletal proteins. MiR-506 represents a potential therapeutic target for PBC patients.

P1183

URSODEOXYCHOLIC ACID INHIBITS HEPATIC CYSTOGENESIS IN EXPERIMENTAL MODELS OF POLYCYSTIC LIVER DISEASE

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Background and Aims: Polycystic liver diseases (PCLDs) are genetic disorders characterized by progressive biliary cystogenesis, which is the main cause of morbidity. Current therapeutic strategies show short-term and/or modest beneficial effects. We and others have recently reported that the hyperproliferation of polycystic cholangiocytes is dependent on the persistent downregulation of the intracellular calcium levels $[Ca^{2+}]_i$ (Perugorria MJ, et al. *Nat Rev Gastroenterol Hepatol*. 2014; *in press*). Thus, pharmacological approaches aimed to normalize the $[Ca^{2+}]_i$ in cystic cholangiocytes have been suggested as potentially beneficial. Here, we investigated the therapeutic value of ursodeoxycholic acid (UDCA) in this condition and the molecular mechanisms of action.
Methods: UDCA was examined *in vitro* and in polycystic (PCK) rats. Hepatic cystogenesis, fibrosis, inflammation and the bile acid content were analyzed in PCK rats treated with UDCA for 5 months. Likewise, the animal motor behavior was evaluated by using the *Open field* test with infrared rays.
Results: Chronic treatment of PCK rats with UDCA for 5 months halts hepatic cystogenesis, fibrosis and inflammation, and improves their motor behavior. As compared to normal animals, PCK rats show increased intrahepatic concentration of bile acids. The *Cyp7a1* mRNA is not different between normal and PCK rat livers and the bile acid concentrations are lower in bile samples collected from PCK than in normal rats. These data suggest that the increased intrahepatic bile acid concentration in PCK rats may be preferentially accumulated in biliary cysts. We found that UDCA decreases the intrahepatic accumulation of cytotoxic bile acids in PCK rats and normalizes the diminished bile acid concentration in bile collected from PCK rats. *In vitro*, UDCA inhibits the hyperproliferation of polycystic human cholangiocytes in a dose-dependent manner without affecting apoptosis rate; this event was associated with increased $[Ca^{2+}]_i$, AKT phosphorylation and diminished ERK1/2 phosphorylation.
Conclusions: UDCA inhibits the hyperproliferation of polycystic human cholangiocytes by raising intracellular $[Ca^{2+}]_i$ that may increase AKT phosphorylation and subsequently inhibits the MAP kinases pathway. UDCA halts the liver disease of an animal model of PCLD, representing a potential therapeutic tool that is currently being evaluated in an international multicenter phase II clinical trial (http://clinicaltrials.gov/show/NCT02021110).

P1184

AGE, BILIRUBIN AND ALBUMIN, REGARDLESS OF SEX, ARE THE STRONGEST INDEPENDENT PREDICTORS OF BIOCHEMICAL RESPONSE AND TRANSPLANTATION-FREE SURVIVAL IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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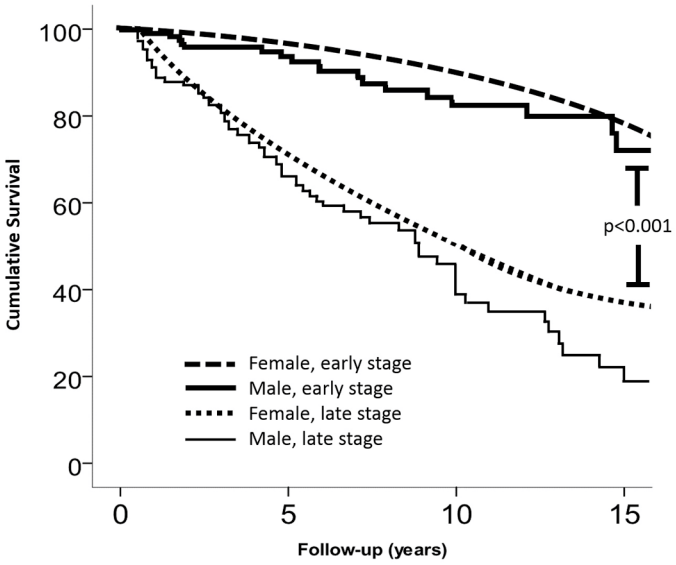
Background and Aims: Primary biliary cirrhosis (PBC) largely affects middle-aged females, thus disease epidemiology in young and male patients is limited. The primary aim was to identify age and sex differences in biochemical response and transplant-free survival using the Global PBC Study Group database.
Methods: A longitudinal study was performed from a well-defined cohort of 4117 patients treated with ursodeoxycholic acid (UDCA). Logistic regression and Cox proportional hazard models were applied, adjusted by center. Biochemical response to UDCA was defined by Paris-1 criteria.
Results: 413 males and 3704 females were included. At diagnosis, males were older (52.9±11.8 vs 56.7±12.6, $p < 0.0001$), with more advanced disease (higher bilirubin, 0.65×ULN vs 0.82×ULN, $p = 0.034$, and more portal hypertension, 22% vs 14%, $p = 0.001$). On univariate analysis, males were less likely to respond than females (60% vs 70%, $p < 0.0001$). After adjusting for age, era of diagnosis, disease stage, log bilirubin, log alkaline phosphatase (ALP) and platelets, sex was no longer an independent predictor of response ($p = 0.852$). Similarly, on univariate analysis for death or liver transplantation, male sex was significant (HR 1.65, 95% CI 1.39–1.95), but this association disappeared after adjusting for age, disease stage, year of diagnosis, log bilirubin, log ALP and response.

Younger patients with early stage disease had a significantly lower response than their older counterparts, regardless of sex (30–39 years: OR 2.24 95% CI 0.99–5.09 vs 70–79 years: 5.92 95% CI 2.44–14.32); patients with moderate/late disease had uniformly poor response to treatment across all age groups.

Conclusions: After adjusting for disease severity, males appear to have comparable response and transplant-free survival as females with PBC. Younger patients with early disease have significantly diminished response compared with older patients at the same stage. Our study suggests that age and disease severity at diagnosis are the most significant independent predictors of response and prognosis in patients with PBC. Early diagnosis and therapeutic intervention is key in these rare subgroups. Young patients with poor UDCA response, particularly with early disease, should be targeted for newer therapies as they have the potential to derive the greatest benefit.



P1185

EFFECTIVENESS OF ADALIMUMAB FOR PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS ASSOCIATED WITH INFLAMMATORY BOWEL DISEASE

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Background and Aims: Primary sclerosing cholangitis (PSC) is a chronic biliary disease with a marked comorbidity with extra-hepatic conditions, mainly inflammatory bowel disease (IBD). The medical treatment for PSC is still disappointing, whereas immunomodulators and biologics have been able to demonstrate efficacy in IBD. *Aim:* To analyse: (1) the natural history of patients with PSC ± associated with IBD; (2) the long-term efficacy of biologics in patients with PSC and concomitant IBD.

Methods: 92 consecutive PSC patients (seen from 1987 to 2014) were included in the study: 50 (54.3%) were males, and 42 (45.7%) females. The mean age at diagnosis was 32.0±14.3 years, and the mean follow-up was 103.8±86 months. Forty-nine patients (53.3%) had associated IBD (38 ulcerative colitis, 10 Crohn's disease, 1 indeterminate colitis). Clinical activity and endoscopic severity were scored according to the CD activity index and Mayo subscore for endoscopy.

Results: Table 1 shows the major events occurred during the follow-up. Three patients with IBD experienced, as second-line treatment, Adalimumab (ADA), an anti-TNFa monoclonal antibody, previous written consensus. Patients were assessed before starting treatment, at month 6 and 12. ADA induction was 160 mg at week 0, and then 80 mg at week 2, while ADA maintenance treatment was 40 mg every 2 weeks. After 6 and 12 months of ADA, a sustained clinical remission of IBD was obtained; a reduction in ALT, GGT, alkaline phosphatase and Mayo PSC score was obtained.

Event	PSC + IBD (N = 49)	PSC − IBD (N = 43)	p
OLTx	7 (14.3%)	7 (16.3%)	0.79
CCA	0	2 (4.6%)	0.13
HCC	2 (4.1%)	0	0.18
Gallbladder cancer	1 (2.0%)	0	0.35
Colorectal cancer	4 (8.2%)	0	0.05
Death	5 (10.2%)	5 (11.6%)	0.83

Conclusions: This is the first study evaluating the efficacy of biologic agents in PSC. Promising results come from ADA for PSC+IBD during a 12 months follow-up. Furthers studies are warranted to investigate the long-term tolerability and efficacy in such patients.

P1186

CHOLESTASIS AND BILE ACIDS INDUCE EXPRESSION OF THE ONCOFETAL MARKER Nope IN ADULT MURINE HEPATOCYTES INDEPENDENT OF FXR

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Background and Aims: Neighbor of Punc E11 (Nope) is strongly expressed in hepatic stem/progenitor cells and in hepatocellular carcinoma but not in terminally differentiated hepatocytes. We here investigated the expression pattern of Nope in adult mice after biliary liver injury.

Methods: Liver tissue was extracted from adult C57Bl/6 mice and $Fxr^{-/-}$ mice 24 hours up to 4 weeks after bile duct ligation (BDL) or after a 1 week diet containing cholic (0.5%) or ursodeoxycholic acid (0.5%). Liver tissue was tested for expression levels of Nope via quantitative RT-PCR. Costainings were performed for Nope in combination with CK19 (biliary), E-cadherin (epithelial) or the canalicular marker dipeptidylpeptidase (DPP) IV.

Results: Bile duct ligation leads to a significantly increasing expression level of Nope (after 1 week 87-fold vs. adult liver, p = 0.00016). Costainings with E-cadherin and DPPIV demonstrate a sinusoidal expression pattern of Nope on hepatocytes, but no expression on CK19-positive cholangiocytes (after 4 weeks 676-fold vs. adult liver). At later stages after BDL, almost all of the hepatocytes stain positive for Nope. In $Fxr^{-/-}$ mice, Nope is expressed without additional biliary injury (28-fold vs. adult liver, p < 0.0007) and shows a further and significant increase after BDL (440- vs. 28-fold, p = 0.03). A diet with cholic (26-fold vs. adult liver, p = 0.002) or ursodeoxycholic acid (8-fold vs. adult liver, p = 0.004) also leads to a significant expression of Nope with a membranous staining pattern on hepatocytes. A diet with hydrophobic acids results in a significantly higher expression of Nope (p = 0.04).

Conclusions: We here report the bile acid-induced expression of the oncofetal marker Nope on adult hepatocytes probably indicating their dedifferentiation. The induction of Nope is not mediated through Fxr but a lack of the receptor leads to a higher expression level probably due to limited compensatory mechanisms of bile acid homeostasis in hepatocytes.

P1187

CD4+ T CELLS FROM PATIENTS WITH PSC EXHIBIT A REDUCED APOPTOTIC SENSITIVITY AND DOWNREGULATION OF PROAPOPTOTIC Bim IN PERIPHERAL BLOOD

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Background and Aims: The immunopathogenesis of the chronic liver disease primary sclerosing cholangitis (PSC) remains largely elusive. Recently, genetic studies indicated that PSC is associated with polymorphisms in the locus encoding for proapoptotic Bim. Bim is crucial for immunological tolerance through induction of apoptosis of activated T cells in response to T cell receptor (TCR) restimulation-induced cell death (RICD) as well as cytokine withdrawal-induced cell death (CWID). We therefore aimed to investigate, whether a defective apoptosis of T cells might contribute to the pathogenesis of PSC.
Methods: A total of 34 patients with PSC, 26 patients with PBC and 32 healthy donors were included in the study. T cells were freshly isolated from peripheral blood and were activated with anti-CD3 and anti-CD28. Induction of apoptosis was triggered by TCR restimulation with anti-CD3 in the presence of IL-2 (RICD) or by IL-2 withdrawal (CWID) for 24 h. Cell loss of CD4+ and CD8+ T cells was quantified by Annexin V staining using flow cytometry. Expression of proapoptotic and antiapoptotic molecules after RICD or CWID was quantified by qRT-PCR.
Results: CD4+ T cells from patients with PSC showed, compared to control groups, a significantly reduced cell loss after induction of apoptosis by RICD (PSC: 20.9% vs healthy: 30.1% vs PBC: 33.6%, $p = 0.0002$) as well as by CWID (PSC: 7.1% vs healthy: 12.1% vs PBC: 12.3%, $p = 0.003$). Cell loss of CD8+ T cells showed no significant differences after RICD or CWID between analysed groups. We next investigated the expression of proapoptotic (Bim, Fas-ligand, Bak) and antiapoptotic molecules (Bcl-2) in T cells. Whereas there was no difference in the expression of Fas-ligand, Bak or Bcl-2, the expression of proapoptotic Bim in T cells from PSC patients was significantly decreased after RICD as compared to control groups (fold induction: PSC: 6.8 vs healthy: 12.9 vs PBC: 12.8, $p = 0.0098$).
Conclusions: Our findings indicate that CD4+ T cells from patients with PSC exhibit a significantly reduced apoptosis sensitivity. A diminished up-regulation of proapoptotic Bim may contribute to this phenotype. Thus, defective apoptosis of activated CD4+ T cells may be part of the immune dysregulation observed in patients with PSC. Future studies must show whether these findings relate to the genetic associations recently described.

P1188

AUTOIMMUNE HEPATITIS (AIH) AND PRIMARY BILIARY CIRRHOSIS (PBC) ALTER THE N-LINKED GLYCOSYLATION PATHWAY

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Background and Aims: Proteins undergo post-translational modifications which can alter their structure and function. *N*-linked glycosylation is one such method of modification. Alterations to glycosylation pathways are involved in diseases such as tumour formation and metastasis. However, not much is known about glycosylation changes in liver disease and injury. The aim of this study was to determine if AIH or PBC alters the *N*-linked protein glycosylation profile of the liver and expression profile of glycosylation-associated genes.
Methods: Liver tissue from non-diseased donors ($n = 10$), AIH patients ($n = 7$) and PBC patients ($n = 7$) was treated with peptide:*N*-glycosidase F to obtain membrane protein *N*-linked glycans which were then analysed using liquid chromatography-electrospray ionization tandem mass spectrometry. Real-time PCR was used to analyse the expression of glycosylation-associated genes involved in the formation of these glycans.
Results: The membrane protein *N*-linked glycan profile of the diseased livers showed that there were less hybrid and complex type glycans and more high mannose type glycans compared to the donor tissue. There was also more fucosylation and less sialylation of the glycan structures in the diseased tissue. The expression of genes known to be involved in the formation of these structures were also dysregulated in the same pattern in which the proteins were affected. MAN1A1 involved in removing mannose residues was downregulated ($P < 0.001$), fucosylation genes FUT4 and FUT8 were upregulated ($P < 0.001$), and sialylation genes ST3GAL2 and ST3GAL6 were downregulated ($P < 0.05$).
Conclusions: Liver cell membrane protein *N*-linked glycan structures were found to be altered in both AIH and PBC compared to donor tissue. This was also reflected in the gene expression data which indicates that the post-translational changes are occurring due to irregularities at the transcriptional level. The pattern of glycosylation also indicates that there is an aberration in the *N*-linked glycosylation pathway where proteins are being processed through the Golgi apparatus to become fucosylated and expressed at the cell surface, but the high mannose type glycan structures indicate that distal changes to the glycan structure are not occurring.

P1189

DECREASED SERUM TWEAK LEVELS ARE PREDICTIVE OF ADVERSE OUTCOME IN PRIMARY SCLEROSING CHOLANGITIS

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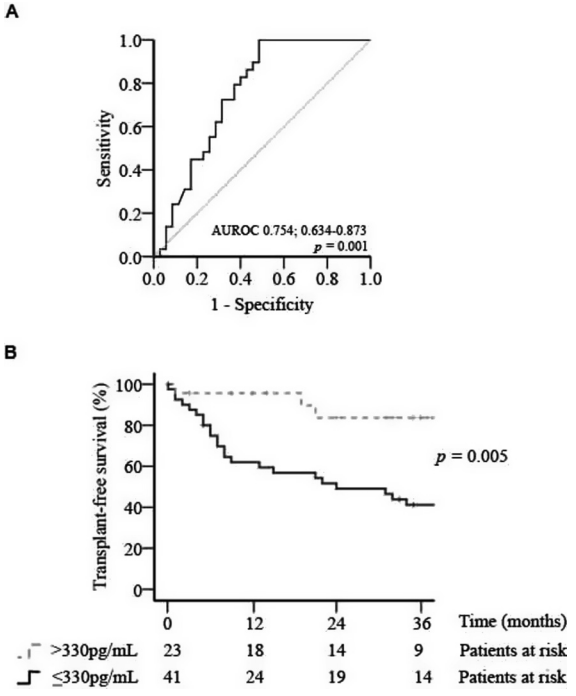
Background and Aims: Primary sclerosing cholangitis (PSC) is a progressive inflammatory cholangiopathy. TNF-like weak inducer of apoptosis (TWEAK) is a mediator of inflammation and a mitogen for ductular reactive cells both of which are prominent features in PSC. Given that robust biomarkers reflective of disease activity and prognosis are lacking in PSC, our aim was to prospectively evaluate circulating soluble (s)TWEAK titre with regard to outcome prediction across a cohort of UK PSC patients.
Methods: Serum samples were obtained from 64 individuals newly diagnosed with PSC between 2010–3 (median age 36; 62.5% male), 24 ulcerative colitis patients and 24 healthy control subjects. sTWEAK was quantified using ELISA and correlated with clinical outcome. Risk-analysis was performed using Cox proportional hazards modelling, logistic regression and Kaplan–Meier survivorship estimates (SPSS v21).
Results: sTWEAK titre was significantly lower in patients with PSC (275 pg/mL, IQR 111–410) compared to healthy controls (942, 885–1044; $p < 0.01$) and individuals with ulcerative colitis alone (1130, 959–1429; $p < 0.0001$). Lower sTWEAK levels when applied as a continuous variable was predictive of rapid progression to liver transplantation/death with high accuracy (AUROC 0.754; Figure 1A). Stratification through a cut point of 330 pg/mL (sensitivity 86%) predicted poorer transplant-free survival in PSC patients with lower titres (unadjusted HR: 4.013; $p < 0.01$; Figure 1B). Additional predictors of poor outcome were elevated

baseline ALP (HR: 1.136), hypoalbuminaemia (HR: 1.164), elevated bilirubin (HR: 1.004), INR >1.2 (HR: 3.497), hyponatraemia (HR: 1.176) and immunosuppression (HR: 3.501) (all $p < 0.05$). However, sTWEAK retained predictive value on multivariable analysis independent of all tested covariates (adjusted HR: 3.628, $p = 0.044$).

Conclusions: Lower sTWEAK levels distinguish patients with PSC compared to those with colitis alone and healthy subjects. Moreover, sTWEAK represents a robust prognostic marker of rapid disease progression. This observation contributes to ongoing efforts serving to refine risk-stratification tools in PSC.



P1190

THE ROLE OF NKT CELLS IN A MOUSE MODEL (NOD.C3C4) WITH SPONTANEOUS BILE DUCT INFLAMMATION

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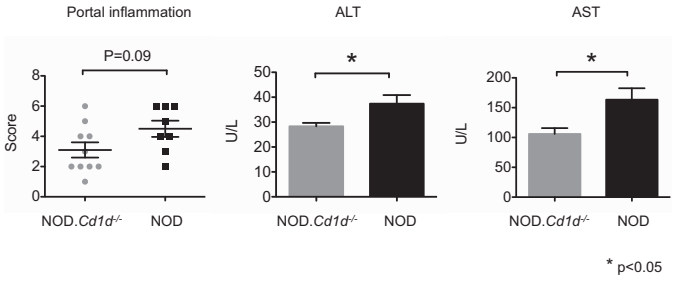
Background and Aims: Natural killer T (NKT) cells are activated by lipid antigens presented by CD1d molecules and represent an important regulatory lymphocyte subset of the liver. The NOD.c3c4 mouse was developed on a NOD background and spontaneously develops biliary inflammation both in extra- and intra-hepatic bile ducts. NOD mice are known to have systemic NKT cell defects (Baxter et al., 1997). We aimed to clarify if NKT cells contribute to disease development in the NOD.c3c4 model.

Methods: The lymphocyte populations from spleens, thymii and livers of NOD.c3c4 mice (n = 3–5) and NOD control mice (n = 3–5) were investigated. Lymphocytes were stained *ex vivo* for NKT cell and activation markers and analyzed by flow cytometry. NOD mice (n = 10) and NOD.*Cd1d*$^{-/-}$ mice (n = 10) were irradiated and injected with NOD.c3c4 bone marrow to create bone marrow chimeras. Three months after bone marrow transfer, liver histology and serum samples were analyzed.

Results: NKT cells were twice as abundant in all three tissues of NOD.c3c4 mice compared to NOD control mice. The NKT cells in the NOD.c3c4 mice displayed an activated phenotype indicated by up-regulation of CD122 in thymus, spleen and liver. Transfer of NOD.c3c4 bone marrow led to a more severe biliary disease in the NOD than in the NOD.*Cd1d*$^{-/-}$ mice. The NOD mice had a trend towards larger biliary inflammatory infiltrates (p = 0.09) and significantly higher serum alanine transferase and aspartate transferase levels compared to *Cd1d*$^{-/-}$mice (p < 0.05 for both comparisons, see figure).

Conclusions: The present study demonstrates that NKT cells are more abundant and show an activated phenotype in liver, spleen and thymus in the NOD.c3c4 model. NOD.c3c4 bone-marrow transfer suggested that CD1d plays a role in development of bile duct inflammation in this model.



P1191

INCREASED IgG4 RESPONSES TO MULTIPLE ENVIRONMENTAL ANTIGENS INDICATE A POLYCLONAL EXPANSION AND DIFFERENTIATION OF PRE-EXISTING B CELLS IN AUTOIMMUNE PANCREATITIS AND IgG4-RELATED CHOLANGITIS

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Background and Aims: Autoimmune pancreatitis and IgG4-related sclerosing cholangitis are the pancreatic and biliary manifestations of a systemic fibro-inflammatory condition (IgG4-related disease; IgG4-RD), characterized by an elevated serum IgG4 concentration and abundant IgG4-positive plasma cells in the involved organs. An important question is whether the elevated IgG4 response is causal, or a mere reflection of immune-regulatory mechanisms of the disease. We sought to investigate if the IgG4 response represents a generalised polyclonal amplification by examining the response to common environmental antigens.

Methods: Serum from 24 patients with IgG4-RD (14 treatment-naïve, 10 treatment-experienced), 9 patients with primary sclerosing cholangitis and an elevated serum IgG4 (PSC-high IgG4), and 18 healthy controls were tested against egg white and yolk, milk, banana, cat dander and serum, peanut, rice and wheat antigens by radioimmunoassay. Serum levels of IgG subclasses and IgE were measured and serum electrophoresis and immunofixation performed.

Results: The concentration of serum total IgG, IgG4, IgE, and to a lesser extent IgG1, was higher in IgG4-RD patients versus healthy controls, as well as in the PSC-high IgG4 patients versus healthy controls. We demonstrated an elevated polyclonal IgG4 response to multiple antigens in patients with IgG4-RD [egg (p = 0.004); milk (p = 0.04); peanut (p = 0.0003); cat dander and serum (p = 0.012); rice and wheat (p = 0.006)] and in PSC-high IgG4 [egg (p = 0.03); cat dander and serum (p = 0.04); rice and wheat (p = 0.01)], compared to healthy controls. There was a strong positive correlation between serum IgG4 and multiple antigen-specific responses. Responses to antigens [banana (p = 0.001), egg (p = 0.039), peanut (p = 0.003), and cat (p = 0.006)] were higher in treatment-naïve compared to treatment-experienced IgG4-RD patients, as were serum total IgG (p = 0.017) and IgG4 (p = 0.001). Serum electrophoresis and immunofixation demonstrated polyclonality in all patients.
Conclusions: This is the first study to show enhanced levels of polyclonal IgG4 to multiple antigens in patients with IgG4-RD. This supports that elevated IgG4 levels reflect an aberrant immunological regulation of the overall IgG4 response, but does not exclude that causality of disease could be antigen-driven.

P1192
AZATHIOPRINE, STEROIDS AND THE PRESENCE OF INFLAMMATORY BOWEL DISEASE BEFORE LIVER TRANSPLANTATION INFLUENCE TRANSPLANT FREE SURVIVAL IN PRIMARY SCLEROSING CHOLANGITIS

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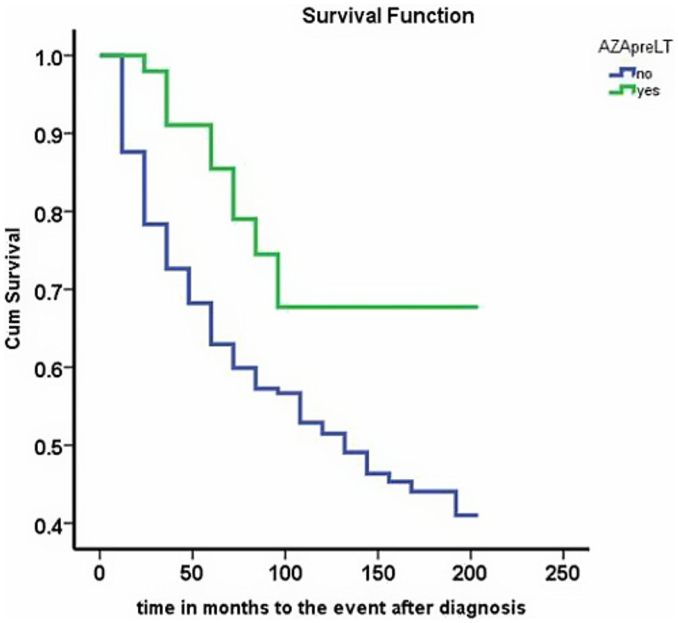
Background and Aims: Primary Sclerosing Cholangitis (PSC) is a chronic, cholestatic liver disease, progressive in the majority of cases. Currently the only therapeutic option is liver transplantation (LT).
Our aim was to assess whether there are any factors affecting the severity of PSC, comparing the course of disease in patients not needing LT and those who died or were transplanted.
Methods: Between 1990 and 2013, 370 consecutive patients with PSC have been diagnosed and followed up. Data was collected retrospectively and Cox regression analysis was used to evaluate all the factors known to affect disease severity: demographics (sex and age at diagnosis), Inflammatory Bowel Diseases (IBD) type [ulcerative colitis (UC), Crohn's disease (CD), indeterminate (IC)] and duration from diagnosis, colonic extent of UC, IBD activity in the last 5 years of follow-up (FUP), IBD treatment during the whole FUP period [steroid, azathioprine (AZA), surgery], severity of disease at last colonoscopy (before last FUP or before LT), prevalence of colorectal dysplasia/carcinoma, PSC duration from diagnosis, PSC severity at last FUP before LT (serum albumin, bilirubin and decompensation), UDCA treatment, cholangiocarcinoma, outcome. FUP was censored at time of LT, death or last FUP.
Results: 234 (63%) male, median age at diagnosis 41 years: 333 patients were diagnosed with PSC, 16 with small duct PSC, 21 with autoimmune overlap. 138 (41%) were transplanted at a median of 40 months from diagnosis. 62 (17%) died at a median of 94 mo from diagnosis. 246 (67%) were diagnosed with IBD: 208 UC, 30 CD, 8 IC. 20 had colon cancer, 13 dysplasia. 66 underwent colectomy.
Cox regression analysis revealed that factors associated with transplant free survival were: AZA use pre-LT (p = 0.012, OR 2.3, 95% CI 1.2–4.4), steroids pre-LT (p = 0.001, OR 4.7, 95% CI 2.2–10.1) and concomitant IBD (p = 0.023, OR 2.11, 95% CI 1.11–3.4). A total of 53 patients were on AZA pre-LT: 7 were transplanted at a median of 62 mo and 5 died at a median of 105 mo from diagnosis. Of 318 not on AZA: 131 underwent LT and 57 died at a median of 44 mo and 82 mo after the diagnosis.
The only factors associated with survival, however, were age at diagnosis <40 years (p = 0.01, OR 1.03, 95% CI 1.01–0.03) and the absence of hepatobiliary malignancy (p = 0.03, OR 0.7, 95% CI 0.5–0.99).
Conclusions: In our cohort of PSC patients, pre-LT use of AZA and steroids significantly prolonged transplant free survival. Patients with concomitant IBD had an increased transplant free survival compared to those without IBD.



P1193
NECROPTOSIS CONTRIBUTES TO THE PATHOGENESIS OF CHOLESTATIC LIVER INJURY

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Background and Aims: Cholestasis is associated with hepatic toxicity and inflammation. In addition, regulated necrosis may be involved in the pathogenesis of inflammatory liver diseases, and kinase activity of receptor interacting protein 3 (RIP3) is key for necroptic signalling. Thus, we aimed to evaluate the role of necroptosis after common bile duct ligation (BDL), a classic experimental model for acute cholestasis and secondary biliary fibrosis, and in human primary biliary cirrhosis (PBC), a cholestatic chronic liver disease.
Methods: BDL or sham surgery was performed in 6–9 animals per group of C57BL/6 wild-type (WT) or RIP3 knockout (KO) mice. Serum and livers were collected 3 and 14 days after BDL. Histology, serum liver enzymes and bilirubin were evaluated. Liver RIP3 and proinflammatory cytokines mRNA were determined by qRT-PCR. Total and soluble/insoluble liver proteins were analysed by Western blot. RIP3 kinase activity was determined *in vitro*. Finally, RIP3 was evaluated in liver specimens of 5 patients with PBC and 5 healthy controls by immunohistochemistry.
Results: BDL resulted in bile duct hyperplasia, multifocal necrosis, fibrosis and inflammatory cell infiltration in mouse liver.

Concomitantly, RIP3 and mixed lineage kinase domain-like (MLKL) were strongly retained in the insoluble protein fraction ($p < 0.05$), which is consistent with the known insoluble amyloid structure of the necrosome. RIP3 mRNA and protein expression, and RIP3 kinase activity were significantly increased ($p < 0.05$), suggesting necroptosis activation. Remarkably, BDL in RIP3 KO mice resulted in a significant decrease in circulating levels of hepatic enzymes and bilirubin (at least, $p < 0.05$), together with decreased expression of proinflammatory cytokines in liver (at least, $p < 0.05$), both at 3 and 14 days. Histological analysis revealed that RIP3 deficiency strongly abrogated liver necrosis and fibrosis induced by BDL. In addition, RIP3 KO decreased RIP1 phosphorylation and caspase-1 activation ($p < 0.05$), with no change in caspase-3/7 activity. Finally, PBC patients showed augmented liver RIP3 expression, when compared with controls ($p < 0.05$).

Conclusions: In conclusion, RIP3-dependent signalling is activated in human PBC and mediates hepatic necroinflammation and fibrosis in BDL mice. As such, RIP3-dependent signalling should be regarded as a potential therapeutic target in cholestatic liver disease.

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P1194

DELAYED BILE ACID UPTAKE WITH METABOLIC CONSEQUENCES IN NA+-TAUROCHOLATE COTRANSPORTING POLYPEPTIDE KNOCKOUT MICE

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Background and Aims: Bile acids (BAs) are metabolic signaling molecules that improve glucose handling and decrease inflammation. We hypothesize that reduced hepatic BA uptake could lead to BA spillover into the circulation, thereby stimulating systemic BA signaling. The Na^+-taurocholate cotransporting polypeptide (NTCP) mediates uptake of conjugated BAs, implying a central function of this basolateral transporter in the enterohepatic circulation of BAs. Here, we investigated conjugated BA dynamics *in vivo* in the first NTCP-knockout mouse model. NTCP was recently also identified as the Hepatitis B Virus (HBV) entry receptor. The HBV entry inhibitor Myrcludex B is described as a potent NTCP inhibitory peptide. Therefore, we tested the specificity of this inhibitory peptide for NTCP *in vivo*.

Methods: We generated a conventional knock-out mouse by deletion of NTCP exon 1. BA kinetics were assessed using tritium-labelled taurocholate (TCA). Mice were fed 0.1% UDCA to challenge the hepatic BA uptake machinery. Bile flow was determined after cannulation of the gall bladder. Expression levels of BA transporters was analyzed by qPCR and Western blot. Mice were imaged by positron emission tomography (PET) to assess binding of gadolinium-labelled Myrcludex B.

Results: NTCP-knockout mice have markedly elevated serum total BA concentrations, mainly composed of conjugated BAs. The body weights of NTCP-knockout mice were significantly reduced directly post-weaning. A subset of knockout mice normalized serum total BA concentrations during maturation, although serum TCA clearance was reduced. The hypercholanemic phenotype was rapidly triggered by UDCA-feeding. Bile flow was slightly reduced, but biliary BA output remained intact. Fecal BA excretion was reduced in hypercholanemic NTCP-knockout mice. However, NTCP-knockout mice showed increased renal BA excretion and reduced *Asbt* expression in kidney. Hepatic uptake of conjugated BAs was potentially affected by downregulation of OATP1A1 and upregulation of OATP1A4. Finally, PET imaging showed a complete abrogation of hepatic binding of Myrcludex B in NTCP-knockout mice.

Conclusions: Chronic NTCP-deficiency causes (episodic) conjugated hypercholanemia, associated with a reduced hepatic BA uptake capacity and renal BA excretion. Finally, Myrcludex B is a specific tool to pharmacologically inhibit NTCP and the NTCP-knockout mouse is an interesting model to study the metabolic effects of conjugated BAs.

P1195

CHARACTERISTIC CHANGES IN BILE ACIDS SYNTHESIS REGULATORY MECHANISMS IN PRIMARY BILIARY CIRRHOSIS. UDCA NON-RESPONSE IS ASSOCIATED WITH HIGHER EXPRESSION OF FGF19

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Background and Aims: Primary biliary cirrhosis (PBC) affects small bile ducts, leading to hepatocellular accumulation of bile acids (BA) and cholestatic injury of the liver. Cholestasis induces adaptive mechanisms protecting the liver against bile salt toxicity. These include regulatory mechanisms of BA synthesis – fibroblast growth factor 19 (FGF19) and farnesoid X receptor (FXR)/orphan nuclear receptor small heterodimer partner (SHP) signaling pathways. Their expression has not yet been studied in PBC. We analyzed expression of FGF19, FGFR4, FXR, SHP, CYP7A1 and phosphorylation of extracellular signal-regulated kinase (P-ERK/ERK) in patients with PBC and searched for their potential relationship with clinical/laboratory findings.

Methods: In total 96 patients with PBC were studied. Serum FGF19, liver biochemistry, Mayo Risk score, health-related quality of life (HRQoL) and 29 bile acids metabolites were analyzed in 51 patients. Liver tissues from non-cirrhotic (n = 24) and cirrhotic (n = 21) patients were used for molecular analysis.

Results: FGF19 mRNA expression was enhanced in non-cirrhotic and cirrhotic tissues (9 fold; $p < 0.0001$ and 69 fold, $p < 0.0001$, respectively) and correlated with fibrosis stage, indicators of cholestasis, Mayo Risk Score and worse HRQoL. Serum levels of FGF19 correlated with worse liver biochemistry, Mayo Risk Score, UDCA non-response and concentration of several bile acids species. Expression of FXR mRNA was increased in non-cirrhotics compared to controls ($p = 0.0001$). Protein levels of FGF19, FGFR4, FXR and SHP were increased in cirrhotic tissue *vs* controls (9 fold, $p < 0.001$; 2 fold, $p = 0.007$; 2 fold, $p = 0.04$; 2 fold, $p < 0.0001$, respectively). This was accompanied by downregulation of CYP7A1 (almost 50% reduction *vs* control, $p = 0.006$). Similar P-Erk/Erk expression levels in PBC and controls were seen, indicating that repression of Cyp7A1 is not modified in MAPK/ERK dependent manner.

Conclusions: PBC induces characteristic changes in liver expression of BA synthesis regulatory molecules. FXR, SHP, FGF19, FGFR4 are increased in PBC, what is accompanied by downregulation of CYP7A1. Lack of response to UDCA is associated with higher expression of FGF19. Whether up-regulation of FGF19 reflects its contribution to compensatory mechanisms in PBC requires further investigation.

P1196

AUTOIMMUNE HEPATITIS WITH T-REGULATORY CELL DEFICIENCY IN THE CONTEXT OF GATA-2 DYSFUNCTION: A NOVEL SINGLE GENE ASSOCIATION

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Background and Aims: To date, only one monogenic disease (AIRE dysfunction) has been associated with autoimmune hepatitis (AIH). We sought to characterise a young woman with AIH as part of a spectrum of immunodeficiency and myelodysplasia.
Methods: Our patient presented to our service aged 27 with hepatitis and ascites. She had a background of lymphoedema in her teens, recurrent superficial skin infections and trilineage cytopaenias requiring repeated transfusion. Investigations revealed IgG at 41.05 g/l, ANA 1:100 and negative viral studies, other than EBV (stable at 10^4-10^5 copies/ml). Liver biopsy showed plasma cells and interface hepatitis consistent with AIH. There was also peribiliary inflammation with granulomata but no mycobacteria, mild steatosis, moderate iron deposition and mild-moderate fibrosis. Staining for other aetiologies including viruses was negative. The International Autoimmune Hepatitis Group score was 21. Corticosteroid treatment induced resolution of ascites and a fall in transaminases, but the patient developed progressive multifocal leukoencephalopathy. Immunosuppression was reduced and transaminses rose again. Peripheral lymphocyte subsets were quantified by flow cytometry, serum Fms-like tyrosine kinase ligand by ELISA and DNA extracted and sequenced.
Results: Sequencing confirmed a novel 1081C>T, R361C mutation in exon 7 of *GATA2*. Monocytes, dendritic, B- and natural killer cells were markedly reduced. Total T-cell numbers were maintained (343.3×10^4/ml; 92–202) but with near absence of $FoxP3^+$ T-regulatory cells (Treg). Serum Flt-3L was elevated (1267 pg/ml; 48–174) consistent with GATA2 dysfunction. The patient was maintained on low dose tacrolimus and steroids but had biochemical evidence of ongoing hepatitis, with worsening anaemia and infections. Stem cell allograft was attempted but failed. Vulval cancer developed secondary to human papilloma virus, and she died from its complications.
Conclusions: Until now, the only monogenic association with AIH has been AIRE dysfunction, although abnormalities in Treg function are proposed. We demonstrate a syndrome consistent with AIH, with near absence of Treg in *GATA2* dysfunction. This is in the context of abnormalities in antigen-presenting and effector cells, and possibly other regulatory mechanisms. Our findings support the hypothesis that deficiencies in regulation, together with unknown environmental triggers (including possible viral infection), may result in an AIH-like illness.

P1197

NATURAL HISTORY OF FATIGUE AND PRURITUS IN PRIMARY BILIARY CIRRHOSIS: IMPLICATIONS FOR THE ROLE OF PRURITUS IN THE AETIOLOGY AND TREATMENT OF FATIGUE

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Background and Aims: Fatigue and pruritus are the most common symptoms associated with Primary Biliary Cirrhosis (PBC) and both can have a significant impact on quality of life. Effective treatments are available for pruritus but not, as yet, for fatigue. It is postulated that pruritus can worsen fatigue, and that itch should be adequately managed prior to assessing fatigue severity however it is not well understood whether an improvement in pruritus is associated with an improvement in fatigue and the degree to which it has an effect. The aim of this study was to investigate whether fatigue and pruritus are stable over time and whether an improvement in pruritus results in an improvement in fatigue.
Methods: 1000 randomly selected patients from the UK-PBC cohort with baseline and follow-up symptom data were used for this study. Symptom data for fatigue was captured using the PBC-40 and intensity of pruritus in the last seven days on a visual analogue scale (VAS). Longitudinal data was captured at entry into the Cambridge genetics study (2009–2011) and follow-up in 2014.
Results: Severity of symptoms at the two time points was not meaningfully different (Figure 1). Scores at the two time points correlated strongly for both symptoms (Figure 2), however there was significantly more variation in the scores for pruritus. A statistically significant association was seen between the delta pruritus scores and the delta fatigue scores at the two time points for an individual (Figure 3) suggesting a change in pruritus (main variable symptom) has a linked effect on fatigue. Of 179 patients who showed a clinically significant worsening in fatigue score, 56 (31%) had worsening of their itch and only 34 (19%) had a worsening of 2 or more VAS units.
Conclusions: Over the follow-up period mean fatigue and pruritus severity remained stable. Baseline and follow-up fatigue and pruritus severity scores showed strong correlations. In the majority of patients fatigue remained static over time, however in contrast, despite pruritus staying the same in net terms there were a significantly greater number of patients whose symptom severity changed (worsening or improving). This is likely to reflect the effects of biological deterioration, development of itch and response to therapy. Fatigue severity change is linked to pruritus severity change in a significant minority of patients suggesting that targeting pruritus is a potentially valuable first step in treating fatigue and should be incorporated into clinical guidelines.

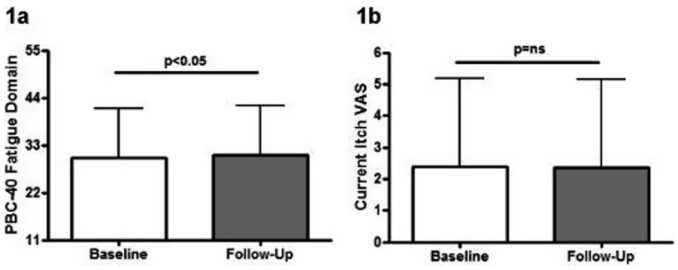

Figure 1.

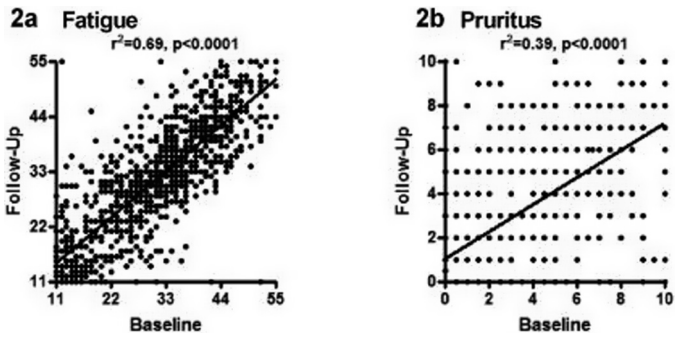

Figure 2.

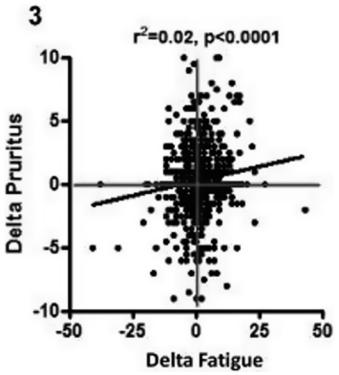

Figure 3.

P1198

HISTOLOGICAL STAGE IS RELEVANT FOR RISK-STRATIFICATION IN PRIMARY BILIARY CIRRHOSIS

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Background and Aims: Risk-stratification in PBC is currently based on the biochemical response to treatment with ursodeoxycholic acid (UDCA) and does not take the stage of the liver disease into account. However, it has been shown that fibrosis and interface hepatitis predict outcome in PBC, independent of the UDCA response. We studied the UK-PBC Research Cohort with the following aims: (1) to identify pre-treatment histological variables that are independently associated with outcome in PBC and (2) to determine whether these variables might improve the accuracy of prognostic models based on UDCA response.
Methods: We analysed liver biopsy reports from PBC patients in the UK-PBC Research Cohort. We classified fibrosis, portal inflammation, interface hepatitis, lobular inflammation, ductopenia, copper-associated protein and steatosis according to their severity. We also classified the reports by Ludwig stage. We defined UDCA response according to the Paris 1 criteria. We defined outcome as liver transplant or liver-related death. Survival curves for each variable were estimated using the Kaplan–Meier method. Cox's proportional hazards models were used to estimate the coefficients for each variable. Akaike's Information Criterion (AIC) was used for variable selection. Correlation coefficient between all continuous variables and fibrosis stage was calculated.
Results: In multivariate analysis, fibrosis [bridging vs. none HR = 3.6 (1.5–8.4), p = 0.004; cirrhosis vs. none HR = 9.5 (3.4–26.8), p < 0.001] and interface hepatitis [focal vs. none HR = 2.6 (1.2–5.7), p = 0.014; widespread vs. none HR = 2.37 (1.0–5.6), p < 0.001] were associated with outcome, independent of UDCA response. The prognostic model consisting of fibrosis, interface hepatitis and UDCA response was more accurate than the model consisting of UDCA response alone (AIC changes from 477.24 to 404.07). Figure 1 shows the plot of survivor function for responder and non-responder to UDCA therapy stratified by the Ludwig stage. Correlation coefficient between platelet count and fibrosis stage was significant but weak (−0.025, p < 0.001).
Conclusions: Fibrosis and interface hepatitis are independently associated with long-term outcome in PBC and improve the accuracy of prognostic models. Liver biopsies may therefore inform risk stratification in PBC. Platelet count correlates to stage of fibrosis – but the correlation may be too weak for platelet count to be used as a surrogate marker. Current recommendations for liver biopsy in PBC should be reviewed.

Level	HR	95% CI		P-value
FIBROSIS				
Variable	**HR**	**25 % CI**	**95% CI**	**P**
none	1			-
portal	1.07	0.39	2.89	0.894
bridging	3.56	1.51	8.36	**0.004**
cirrhosis	9.49	3.36	26.83	**<0.001**
INTERFACE HEPATITIS				
none	1			
focal	2.62	1.12	5.66	**0.014**
widespread	2.37	1.01	5.59	**0.048**
UDCA response	3.60	2.3	5.6	**<0.0001**

Figure 1. Histological variables independently associated with LT or liver-related death.

P1199

TACROLIMUS IS SAFE AND EFFECTIVE IN PATIENTS WITH RESISTANT TYPE 1 AUTOIMMUNE HEPATITIS

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Background and Aims: Autoimmune hepatitis (AIH) is an immune mediated liver injury related to loss in self-tolerance requiring long term immunosuppressive therapy. Most patients respond to treatment with prednisolone and azathioprine but second line immunosuppressive therapy may be required in those who fail to respond. Tacrolimus is a potent inhibitor of T cell activation used to prevent graft rejection in organ transplantation.
Aims: To investigate the clinical efficacy and safety of tacrolimus in patients with refractory AIH.
Methods: Data were collected retrospectively between 1997 and 2014 from 19 patients being treated in two tertiary referral liver units' centres in the United Kingdom and Germany. Biochemical, immunological and MELD scoring improvement was assessed.
Results: The majority of patients were Caucasian (84%) and female (63%); median simplified AIH score was 7 (range: 4–8) and 16% were cirrhotic at presentation. The median age at diagnosis was 30 years (Range: 16–70). Most (95%) were treated with prednisolone and azathioprine [AZA] as first line treatment prior to tacrolimus therapy. The median time of starting tacrolimus from diagnosis was 2.5 years. 63% of patients were started on tacrolimus due to incomplete remission or flare of AIH despite apparent adherence

to therapy; 21% were intolerant of first line treatment with AZA and 11% were started at initial diagnosis of AIH. Median duration of treatment was 24 months and median dose of tacrolimus was 2 mg per day. The majority of patients were on prednisolone (58%) and azathioprine (42%) in addition to tacrolimus. There was a significant improvement in aspartate transaminase levels ($p < 0.05$) at 6, 12 months and at the latest follow up. Bilirubin fell significantly at 12 months and latest follow up ($p < 0.05$) and IgG at the latest follow up. The median model for end stage liver disease score (MELD) pre treatment was 8 which was stable at 12 months (median 7). No significant side effects or renal dysfunction were reported during the treatment period. All patients are alive during the follow up although one underwent liver transplantation. Tacrolimus treatment was discontinued in 9 patients because of a failure to respond and of these, 4 patients had developed PSC/AIH overlap on follow up liver biopsies and magnetic resonance cholangiopancreatography (MRCP).

Conclusions: Tacrolimus therapy in patients with resistant type 1 AIH is safe, generally effective and improves biochemical and MELD score.

P1200
THE EFFECT OF AZATHIOPRINE ON THE RISK OF MALIGNANCY IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS: A LONG-TERM MULTICENTER STUDY

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Background and Aims: Primary sclerosing cholangitis (PSC) is a chronic progressive liver disease of unknown etiology. One of the major clinical challenges in PSC is the development of hepatobiliary malignancy. Approximately 15% of patients develop cholangiocarcinoma (CCA), which is associated with a poor prognosis. In addition, the risk of colon carcinoma and gall bladder cancer is increased. Many patients are treated with azathioprine. The effect of azathioprine, however, on the risk of hepatobiliary malignancy has not been evaluated so far. Both an enhanced as well as a lower risk for CCA development under immunosuppressive treatment could be expected. We therefore investigated the risk of hepatobiliary malignancy in a large multicenter cohort of patients with PSC with respect to azathioprine treatment.

Methods: We performed a retrospective study in a cohort with well-defined PSC from three large tertiary care centers from Germany and Norway. With respect to risk calculation, we only analyzed malignancy development, which was observed after the first diagnosis of PSC, and considered azathioprine treatment, when administered between PSC diagnosis and one of the endpoints: malignancy development, liver transplantation, death or lost to follow-up. Statistical analysis was performed using an univariate Cox proportional hazard model under the assumption of competing risks of different endpoints.

Results: 683 patients (472 male) were included into the study. 484 (71%) patients had concomitant inflammatory bowel disease, 89 (13%) an overlap syndrome with autoimmune hepatitis. Within a median observation time of 9.4 years, in 41/683 patients (6%) a CCA was diagnosed. CCA developed in 3/118 (2.5%) of patients who had been treated with azathioprine as compared to 38/565 (6.7%) of patients not treated with azathioprine. Using the univariate hazard model this difference was not statistically significant. Also, azathioprine treatment did not seem to affect the risk of gallbladder (0.6% in the whole group) or colorectal cancer (1% in the whole group).

Conclusions: Based on this retrospective multicenter cohort including a large number of patients and a long observation period, treatment with azathioprine did not significantly affect the risk of hepatobiliary malignancy in patients with PSC. Therefore, in patients with PSC and concomitant inflammatory bowel disease or autoimmune hepatitis, the treatment with azathioprine should not be withheld.

P1201
LONG-TERM THERAPY WITH BEZAFIBRATE AND URSODEOXYCHOLIC ACID IS INSUFFICIENT FOR PREVENTING DISEASE PROGRESSION IN PATIENTS WITH ADVANCED PRIMARY BILIARY CIRRHOSIS

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Background and Aims: It has been observed that treatment with fibrates is effective to normalize liver biochemistries in patients with primary biliary cirrhosis (PBC) and suboptimal response to ursodeoxycholic acid (UDCA). However, there are no consistent data regarding the long-term course and outcome in these patients, and therefore, we have assessed the effects of the combined treatment with UDCA and bezafibrate for a long period of time.

Methods: 48 patients (45 female) with PBC treated with UDCA for a mean 9 years and alkaline phosphatase (AP) levels (>1.5 xUNL). Patients were treated with bezafibrate (400 mg/d) plus UDCA (13–16 mg/kg/d), and therapeutic response and baseline characteristics, clinical and biochemical changes and prognosis after long-lasting therapy was assessed. The full therapeutic response was defined as AP normalization.

Results: After a median of 38 months of treatment, patients were divided into two groups according to AP normalization. 26 patients (54%) normalized AP (median 4 months). In these patients, baseline jaundice (0% vs 18%, $p = 0.03$), pruritus (19% vs 67%, $p = 0.007$) and liver stiffness (6.9 ± 0.5 vs 13.2 ± 2.5 kPa, $p = 0.007$) was lower, and age was higher (57.2 ± 1.7 vs 49.8 ± 2.4 years, $p = 0.01$) than in patients who remained with elevated AP. These patients also had lower transaminase (ALT), AP, gamma glutamyl transferase (GGT), bilirubin, albumin and cholesterol and higher than patients without complete response. During the assessment period, partial or complete itching relieve was observed in all cases, regardless of the biochemical AP response. Moreover, improvement in all biochemical parameters was observed in the patients who normalized AP, while these effects were less prominent in the patients without full AP response. During follow-up, 5 patients, all of them without AP normalization (23%) developed events of disease progression (one patient died, 3 were transplanted or fulfilled criteria for transplant, and one patient developed a hepatocellular carcinoma).

Conclusions: The long-term treatment with UDCA and bezafibrate results in excellent response in more than half of the cases, and it is associated with a complete or partial itching relieve. The absence of complete response was observed in patients with advanced disease who remain at risk of developing severe events. Consequently, the combined treatment is especially effective in patients with lower fibrosis and severity of cholestasis.

P1202

DEFINITION AND COMPARATIVE ANALYSIS OF THE T CELL RECEPTOR REPERTOIRE IN PATIENTS WITH PRIMARY SCLEROSING CHOLANGITIS

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Background and Aims: Primary sclerosing cholangitis (PSC) is a chronic liver disease characterized by immune-mediated biliary injury and a clear HLA association. T cell infiltration is characteristic of the PSC lesions, but their target antigen specificity is still unknown. We aimed to characterize the T cell receptor (TCR) beta chain complementarity-determining region 3 (CDR3) of liver-infiltrating T cells to assess if disease-specific TCR repertoires could be identified in the livers of patients with PSC relative to controls with primary biliary cirrhosis (PBC) or alcoholic liver disease (ALD).
Methods: Genomic DNA (400 ng) from PSC (n = 20), PBC (n = 10) and ALD (n = 10) was extracted from liver tissue sampled at liver transplantation and subjected to high-throughput sequencing by ImmunoSEQ pipeline (Adaptive Biotechnologies Corp, Seattle, WA). Genomic DNA from the same samples was used for *HLA-DRB1* and *HLA-B* typing.
Results: In PSC cases a higher number of productive unique TCR beta sequences (clones in which the entire TCR gene is expected to translate to a functional protein) was detected (16,544±1,000) (mean±SEM), compared to PBC (14,264±1,754, $P = 0.4$) and ALD (8,123±1,451, $P = 0.0002$). Assessment of TCR clonal frequency distribution revealed an overall lower clonality of PSC samples (0.134±0.007), compared to PBC (0.153±0.013) and ALD (0.146±0.013), further suggesting a higher T cell diversity in PSC. Nevertheless, eight TCR beta sequences were uniquely shared in 30–40% of PSC cases (Table 1), in frequencies ranging from 2×10^{-4}% to 4×10^{-2}%, and were completely absent from PBC and ALD disease control groups. Different V family genes were used to create these eight amino acid sequences, supporting antigen-driven selection.
Conclusions: Our data suggest the presence of a highly diverse TCR repertoire in PSC patients, however, also demonstrate eight clones uniquely found in PSC but not in liver disease control groups. Refined assessments are needed to distinguish non-specific phenomena (e.g. end-stage liver disease and biliary bacterial colonization) from potentially antigen-specific T cell expansions.

P1203

EFFECT OF IMMUNOSUPPRESSIVE DRUGS ON THE KINETICS OF CO-INHIBITORY MOLECULE EXPRESSION AND PRO-INFLAMMATORY CYTOKINE PRODUCTION BY EFFECTOR T CELLS IN AUTOIMMUNE HEPATITIS

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Background and Aims: Immunosuppressive drugs (ISDs) are the mainstay of treatment for autoimmune diseases, including autoimmune hepatitis (AIH). Little is known about the effects that ISDs have on the kinetics of effector T cell (Teff) inhibitory molecule expression or pro-inflammatory cytokine production in the context of autoimmunity. We aimed to investigate the effect of ISDs on Teff activation-induced expression of co-inhibitory molecules and pro-inflammatory cytokines.
Methods: $CD4^{pos}CD25^{neg}$ Teff cells were isolated immunomagnetically from the peripheral blood of eight healthy subjects (HS) and six newly-diagnosed ANA/SMA^{pos} treatment-naïve AIH patients. Teff cells were stimulated polyclonally with or without rapamycin, prednisolone, FK-506, mycophenolic acid, 6-mercaptopurine or cyclosporine. Expression of the co-inhibitory molecules TIM3, PD1 and CTLA4 and of the pro-inflammatory cytokines IL17, IFNγ, and TNFα was assessed by flow-cytometry.
Results: In HS and AIH, TIM3 expression increased over 96 hrs, without ISDs exerting a significant effect ($p < 0.01$ for all comparisons). TIM3 expression peaked more quickly in AIH compared to HS, with higher levels present at 48 hrs ($p < 0.001$ by two-way ANOVA). PD1 expression was higher in AIH compared

Table 1 (abstract P1202).

PSC specific clones	Number of samples detected (Total = 20)		HLA haplotype
CASSFTGTDTQYF	6	HLA-DRB1	03:01, 15:01; 01:01, 03:01; 01:01, 13:01; 07:01, 11:04; 03:01, 11:04; 07:01, 13:01
		HLA-B	08:01, 04:01; 07:02, 08:01; 15:01, 37:01; 18:01, 57:01; 07:02, 38:01; 44:02, 57:01
CASSPFSYEQYF	6	HLA-DRB1	07:01, 15:01; 03:01, 15:01; 13:02, 15:01; 03:01, 03:01; 01:01, 13:01; 11:01, 15:01
		HLA-B	07:02, 13:02; 08:01, 04:01; 07:02, 50:01; 08:01, 08:01; 15:01, 37:01; 03:01, 15:18
CASSDTSGGADTQYF	6	HLA-DRB1	03:01, 15:01; 11:01, 13:01; 04:01, 07:01; 03:01, 07:01; 01:01, 15:01; 11:01, 15:01
		HLA-B	08:01, 04:01; 35:03, 51:01; 15:01, 44:03; 08:01, 44:03; 07:02, 51:01; 03:01, 15:18
CASSELAGGPETQYF	6	HLA-DRB1	07:01, 15:01; 03:01, 15:01; 03:01, 07:01; 07:01, 11:04; 07:01, 13:01; 11:01, 15:01
		HLA-B	07:02, 13:02; 08:01, 04:01; 03:01, 44:03; 18:01, 57:01; 44:02, 57:01; 03:01, 15:18
CASSGTSGGADTQYF	6	HLA-DRB1	03:01, 15:01; 04:01, 07:01; 01:01, 15:01; 07:01, 11:04; 03:01, 11:04; 11:01, 15:01
		HLA-B	08:01, 04:01; 15:01, 44:03; 07:02, 51:01; 18:01, 57:01; 07:02, 38:01; 03:01, 15:18
CASSEYSNQPQHF	7	HLA-DRB1	03:01, 15:01; 13:02, 15:01; 01:01, 03:01; 13:01, 15:01; 04:01, 07:01; 03:01, 07:01; 01:01, 13:01
		HLA-B	07:02, 03:01; 07:02, 50:01; 07:02, 08:01; 08:01, 39:01; 15:01, 44:03; 14:02, 18:01; 15:01, 37:01
CASSLGSGAWLTF	7	HLA-DRB1	07:01, 15:01; 13:02, 15:01; 03:01, 03:01; 01:01, 03:01; 01:01, 03:01; 01:01, 15:01; 07:01, 13:01
		HLA-B	07:02, 13:02; 07:02, 50:01, 03:01, 08:01; 07:02, 03:01; 08:01, 39:06; 07:02, 51:01; 44:02, 57:01
CASSPGQGEGYEQYF	8	HLA-DRB1	03:01, 15:01; 03:01, 03:01; 01:01, 03:01; 03:01, 03:01; 13:01, 15:01; 01:01, 03:01; 03:01, 07:01; 07:01, 13:01
		HLA-B	07:02, 03:01; 08:01, 08:01; 07:02, 08:01; 08:01, 18:01; 08:01, 39:01; 08:01, 39:06; 08:01, 44:03; 44:02, 57:01

to HS (at 48 hours; $p < 0.0001$ and 96 hrs; $p = 0.0026$ by two-way ANOVA). In AIH patients, PD1 continued to increase between 48 and 96 hrs ($p < 0.05$) but this was prevented by ISDs. CTLA4 expression increased over time to similar extents in AIH and health, with little effect of ISDs.
Cytokine expression tended to increase rapidly by 48 hrs, falling again by 96 hrs. In contrast to HS, IFNγ production continued to increase between 48 and 96 hrs in AIH patients ($p < 0.05$). ISDs prevented this increase, with cyclosporine significantly lowering IFNγ expression ($p < 0.01$).
Conclusions: Co-inhibitory molecules and pro-inflammatory cytokines display distinct patterns of activation-induced expression. PD1 and IFNγ increases are restrained by ISDs in AIH, suggesting a novel mechanism of action of these drugs.

P1204

GENOMIC T CELL RECEPTOR SIGNATURES IN MATCHED GUT, LIVER AND BLOOD SAMPLES FROM PRIMARY SCLEROSING CHOLANGITIS PATIENTS

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Background and Aims: Primary sclerosing cholangitis (PSC) is a chronic liver disease of unknown etiology where the majority of patients have concurrent inflammatory bowel disease (IBD). Previous studies have reported that gut-specific addressins and chemokines are also involved in the recruitment of mucosal T cells to the liver of PSC patients. We hypothesized that common antigenic determinants form the basis of this shared T cell response and aimed herein to assess if T cell receptor (TCR) beta sequences previously detected in liver tissue of PSC patients are also detected in matched gut and blood samples.
Methods: Genomic DNA was isolated from liver tissue obtained at liver transplantation, gut biopsies and blood from ten Norwegian PSC patients. All PSC patients had a concurrent diagnosis of IBD (nine had ulcerative colitis, one had Crohn's disease). Sequencing of the TCR beta chain complementarity-determining region 3 (CDR3) of all specimens was performed using the ImmunoSEQ pipeline (Adaptive Biotechnologies Corp, Seattle, WA).
Results: Detection rates in liver tissue, gut biopsies and blood for each of the previously reported PSC specific TCR beta sequences are given in Table 1. All previously reported PSC specific TCR beta sequences were detected in the present patient panel, mostly at lower rates in liver (0–5 patients) and gut (0–4 patients) than in blood (2–9 patients). Assessment of the general overlap between paired liver and gut samples for the 200 most frequent sequences in the TCR repertoire of each sample revealed a mean of 17.3% TCR beta sequences shared between the liver and gut of each patient (with a range from 4.0% to 34.0%).
Conclusions: Previously reported PSC specific TCR beta sequences were detected in blood, liver and gut of the present independently sampled patient panel. The relationship between the observed overlap of T cell specificities and the shared mechanisms of liver and gut T cell recruitment mechanisms warrants further study.

TCR beta sequence	N = 10 Identified in liver # of Norwegian PSC patients	N = 10 Identified in gut # of Norwegian PSC patients	N = 10 Identified in blood # of Norwegian PSC patients
CASSPGQGEGYEQYF	2	1	3
CASSLGSGANVLTF	5	2	9
CASSDTSGGADTQYF	1	0	2
CASSGTSGGADTQYF	3	1	2
CASSFTGTDTQYF	0	1	3
CASSPPSYEQYF	1	2	8
CASSELAGGPETQYF	1	4	3
CASSEYSNQPQHF	1	1	3

P1205

COMPREHENSIVE ANALYSES OF AUTOIMMUNE HEPATITIS TYPE 1 ASSOCIATED mRNA, lncRNA, AND miRNA IN CD4+ T CELLS TARGETED BY PREDNISOLONE TREATMENT

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Background and Aims: Prednisolone (PSL) is commonly used to treat autoimmune hepatitis (AIH) type 1. Although the progression of disease becomes mild in almost all patients treated with PSL, some patients resist the treatment and require additional immunosuppressors. Mechanism of PSL treatment for AIH is still not fully understood. Thus, we analyzed the dynamics of gene expression by PSL treatment including messengerRNA (mRNA), long non-codingRNA (lncRNA), and microRNA (miRNA) in CD4+ T cells to reveal the mechanism of PSL treatment for AIH.
Methods: Clinically and pathologically diagnosed 2 naïve AIHs (nAIH), 7 AIHs in remission with PSL (rAIH), and 7 healthy controls, who agreed to provide samples with written informed consent, were enrolled in this study. This study was approved by the institutional review board. Total RNA was extracted from CD4+ T cells purified from peripheral blood. The comprehensive analysis of the genes was undergone using microarrays. Differentially expressed genes were compared among 3 groups using the fold change (FC) cut-off and the ANOVA. The data were classified by hierarchical clustering to extract the PSL targeted genes. Then correlation analysis was performed to extract the genes correlating with the liver injury.
Results: Microarray study showed that 1,072 mRNAs, 128 lncRNAs ($p < 0.05$, FC >1.5), and 17 miRNAs ($p < 0.05$, FC >1.2) were differentially expressed among 3 groups. By the hierarchical clustering, each group was clearly distinguished by mRNA and lncRNA expression, and their expression profiles were classified into 8 clusters. One mRNA cluster consisted of differentially expressed mRNAs, which decreased from nAIH to rAIH and became close to healthy control by PSL treatment. Inflammation associated 6 cytokines and 3 chemokines were extracted from this cluster. The expression of cytokine/chemokine was strongly correlated with each other and the value of ALT. Moreover, also 7 lncRNA and 7 miRNA were correlated with cytokine/chemokine expression and the value of ALT.
Conclusions: PSL treatment changed the mRNA/lncRNA/miRNA expression profile of CD4+ T cell in AIH. Certain differentially expressed mRNAs/lncRNAs/miRNAs were identified to be key players in the pathogenesis of AIH targeted by PSL treatment.

P1206

ANTI-INFLAMMATORY AND DIRECT ANTIFIBROTIC EFFECT OF ORAL HEPATOTROPIC DPP4 INHIBITORS IN MODELS OF NASH AND BILIARY FIBROSIS

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Background and Aims: Non-alcoholic steatohepatitis (NASH) is characterized by steatosis, lobular inflammation and progressive parenchymal fibrosis. Anti-inflammatory and especially antifibrotic therapies for NASH are urgently needed. Glucagon like peptide 1 (GLP-1) is an attractive molecule for the treatment of insulin resistance. GLP-1 is rapidly inactivated by cell surface dipeptidyl peptidase-4 (DPP-4). Therefore we studied the effect of two indirect GLP-1 agonists, hepatotropic DPP4-inhibitors, on liver inflammation and fibrosis in models of NASH and biliary fibrosis.
Methods: Linagliptin and Sitagliptin were administered daily by oral gavage to Mdr2KO mice and to C57BL/6mice fed a methionine and choline deficient (MCD) diet for 4 and 6 weeks. Hepatic fibrosis was assessed by morphometric analysis of Sirius red stained collagen and measurement of hydroxyproline content. Serum biochemistries were determined by an autoanalyzer, and hepatic inflammation and fibrosis were assessed by semiquantitative immunohistochemistry. Fibrosis and inflammation related transcript levels were measured by quantitative real-time polymerase chain reaction (qPCR). Ex vivo analysis of hepatic inflammatory cells was done by FACS.
Results: In the 4 week treatment group only 50 mg/kg/day of Linagliptin lowered serum ALT, AST, ALP and LDH and also decreased fibrosis and inflammation related gene expression (aSMA, TIMP-1, TGFβ1, MMP-13, CD68 and TNF α) compared to vehicle-treated controls. However, after 6 weeks' treatment, both Linagliptin and Sitagliptin reduced hepatic collagen accumulation, and also decreased fibrosis and inflammation related gene expression (aSMA, TGFβ1, TNF α). The expression of CD68, F4/80, Caspase 3, as determined by semiquantitive immunohistochemistry, was significantly suppressed. There was a decrease of hepatic $CD11b^{+}Ly6C^{+}$high (proinflammatory monocytes/macrophages) and of total F4/80+ cells (macrophages, Kupffer cells) when compared to mice that received the MCD diet without treatment. In Mdr2KO mice 10 and 50 mg/kg/day of Linagliptin significantly decreased procollagen α1(I), TGFβ1, TIMP-1, MMP-8 transcript levels, but increased putatively anti-fibrotic MMP-9 and -13.
Conclusions: In mice fed the MCD diet for 6 weeks, the DPPIV inhibitors Linagliptin and Sitagliptin significantly decreased inflammation and apoptosis. Linagliptin and Sitagliptin attenuated liver injury and fibrosis through inhibition of inflammatory and apoptosis pathways that are prevalent in human NASH.

P1207

FATIGUE IN PRIMARY BILIARY CIRRHOSIS: SIMILAR PREVALENCE TO THE POPULATION FROM THE SAME GEOGRAPHIC AREA, AND ASSOCIATION WITH COMORBIDITIES AND SEVERITY OF CHOLESTASIS

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Background and Aims: The pathogenesis of fatigue in patients with primary biliary cirrhosis (PBC) is unknown, and previous studies are uncertain regarding the specificity of this symptom and the association with comorbidities. It has also been suggested that the prevalence of this symptom varies by geographic area. Therefore, we have assessed the prevalence, severity and potential risk factors in a series of patients with primary biliary cirrhosis as compared with a age-matched control group of the same geographical area.
Methods: A multidomain disease-specific quality of life responses and clinical findings questionnaire for PBC (PBC-40) was distributed to a consecutive series of 182 patients and in an age mactched group of 48 women of similar age group. Clinical, laboratory, histological, duration and biochemical response to ursodeoxycholic acid (UDCA), and comorbidities were recorded in 108 PBC patients.
Results: PBC-40 scores were significantly different between patients and controls for symptom (18.7±4.9 vs 16.4±4.2. $p = 0.003$), itching (4.5±2.4 vs 3.3±0.6, $p < 0.0001$), cognitive (11.9±5.9 vs 9.4±4.2, $p = 0.007$) and social (18.9±7.4 vs 16.7±4.0, $p = 0.05$) domains, but were similar for fatigue (23.3±11 vs 23.3±8.8, p:ns) and emotional (7.2±3.5 vs 6.8±2.4 p: ns) domains. Depending on the fatigue severity patients were divided into two groups: severe/moderate fatigue and no/mild fatigue. There were no significant differences in age, sex, biochemical variables, duration of UDCA treatment and histology at diagnosis between the two groups. Patients with severe/moderate fatigue had lower hemoglobin levels (12.4±1.9 vs 13.1±1.3 g/l, $p = 0.002$), higher levels of total bilirubin (1.1±0.5 vs. 0.3±0.5 mg/dl, $p = 0.001$) and (gGT 210±45 vs 129±20 U/ml, $p = 0.005$) as compared with patients with no/mild fatigue. There was no relationship between fatigue and hypothyroidism, sicca syndrome or with response to UDCA. Diagnosis or therapeutic intervention for depression was found in 18 of 78 patients (23%), and depression was significantly associated with fatigue (OR: 5.8, 95% CI: 1.9–17.9, $p = 0.003$).
Conclusions: Fatigue is a common manifestation in but similar to the control population of the same area. Fatigue is related to the severity of the disease, and comorbidities such as depression and anemia, but it is independent of the disease duration and therapeutic response to ursodeoxycholic acid.

P1208

ANALYSIS OF HUMORAL IMMUNE RESPONSE IN A NEW ANIMAL MODEL OF AUTOIMMUNE HEPATITIS: NEW ANTIGENS IDENTIFICATION

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Background and Aims: Autoimmune Hepatitis (AIH) is a chronic disease characterized by the immuno-dependent destruction of hepatocytes, because of the loss of immunologic tolerance to autoantigens. Therapeutic alternatives are limited to control inflammation with immunosupressive and immunomodulatory drugs. The molecular mechanism of the trigger of the immune attack is poorly understood. The progress in this field is critical to develop new treatments, being necessary animal models that represent clinical, histological and serological manifestations of human AIH. In our laboratory, we have developed an animal model that mimics main characteristics seen in humans. One of our aims has been to look forward new antigens in this new model.
Methods: The model of AIH has been established through the administration of an adeno-associated virus (AAV) that express hepato-specifically IL-12 (AAV-IL12), triggering an immune response against hepatic antigens, harming liver parenchyma. Vectors AAV-IL12 and AAV-Luc (as control) were injected intravenously in C57BL/6 mice, that were sacrificed at day 60. Histopatological analysis was done, certifying the disease

development. Reactivity of serum auto-antigens was done by using a protein array, NAPPA (Nucleic Acids Programmable Protein Arrays), containing >4000 proteins and its corresponding controls, being identified different serum profiles between both situations.
Results: At 60 days, livers coming from IL-12 mice showed a mononuclear infiltrate, piecemeal necrosis, apoptotic hepatocytes and liver fibrosis whether controls were normal. IL-12 mice serum recognised many proteins that weren't recognised by control mice serum. Proteins that reacted more and were expressed in the liver in AIH mice were selected and are being validated individually.
Conclusions: The identification of new hepatic antigens involved in AIH, can open doors in the development of new and specific therapeutic strategies as the induction of tolerance against these antigens.

P1209
CORONARY MICROVASCULAR DYSFUNCTION IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS WITHOUT METABOLIC SYNDROME: A HINT FOR THEIR INCREASED CARDIOVASCULAR RISK

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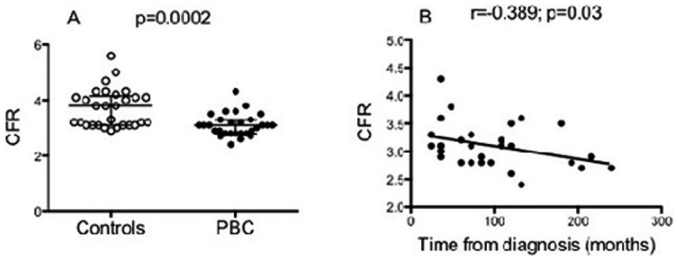
Background and Aims: Primary biliary cirrhosis (PBC) is characterized by a long natural history and a low incidence of cardiovascular events despite high serum cholesterol levels. Metabolic syndrome may increase the cardiovascular risk, however. Coronary flow reserve (CFR) is widely used to examine the integrity of coronary microvascular circulation and it is well recognized as a predictor of cardiovascular outcome. *Aim:* To evaluate the risk of coronary microvascular dysfunction in patients with PBC without metabolic syndrome.
Methods: 29 PBC patients (27 F, aged 56±11 years) without clinical evidence of heart disease and without metabolic syndrome, and 29 sex- and age-matched healthy controls underwent CFR by transthoracic Doppler echocardiography (TDE). Coronary flow velocity in the left anterior descending coronary artery was detected by TDE at rest and during iv. adenosine infusion. CFR was the ratio of hyperaemic diastolic flow velocity (DFV) to resting DFV.
Results: The median time between the onset of symptoms and CFR assessment was 7 years (interquartile range 3–10.5 years). In PBC patients CFR was significantly lower than in controls (3.1±0.3 vs 3.8±0.4, $p < 0.0002$) (Fig. A). CFR was inversely related to the time from diagnosis ($r = -0.389$, $p = 0.03$) (Fig. B) and age ($r = -0.370$, $p = 0.04$), and positively correlated with TSH ($r = 0.411$, $p = 0.03$). No relationship was found between CFR and LDL-cholesterol.
Conclusions: CFR is impaired in PBC patients and is correlated with the length of disease, suggesting a negative effect of PBC on coronary microcirculation that may contribute to the increased cardiovascular risk, even in patients without metabolic syndrome.



P1210
IMPACT OF SPECIALIST PATHOLOGY REVIEW OF LIVER BIOPSIES ON THE FINAL DIAGNOSIS AND PATIENT MANAGEMENT

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Background and Aims: It is standard UK practice for all patients with a malignant diagnosis to be discussed at multidisciplinary team (MDT) meetings, which includes histopathological review, prior to planning treatment. Studies have shown a discrepancy rate of 7–15% between the original diagnosis and specialist centre review. Few studies have explored the potential impact on patient management of such a review for non-malignant pathologies.
This study reviewed all liver biopsies referred to our centre, a university teaching hospital and transplant centre, to determine (a) the extent of agreement with the original report; (b) the predicted impact on patient management; (c) the main areas of diagnostic discrepancy.
Methods: All native and transplant liver biopsies for focal lesions and non-lesional assessments referred for review from 2010 to 2013 were included. The extent of agreement with the original diagnosis and probable impact on clinical management were obtained from the histopathology report, with discussion with Consultant Hepatologists as required.
Results: A total of 1333 liver biopsies met inclusion criteria. The majority, 65%, were referred for specialist advice; 24% were reviewed due to transfer of care or joint clinical management, including transplant assessment; and 11% for MDT discussion. In 786 cases, 59%, there was a difference in interpretation compared with the original report, 67% of which were predicted to result in some change in patient management. This was most frequent in cases referred specifically for second opinions, although 28% cases reviewed for a MDT meeting and/or transfer of care showed differences likely to change treatment.
In 64% with a final diagnosis of chronic biliary disease, and over half where the major pathology was autoimmune hepatitis, acute hepatitis, vascular/architectural abnormalities, or a benign lesion, specialist review was associated with a potential alteration of management. Although agreement was better for chronic viral hepatitis, fatty liver disease and malignant lesions, in 24–36% of cases altered management was still likely after the biopsy review.
Conclusions: A specialist pathology review of liver biopsies may result in changed patient management in almost 40% of cases. Although relevant to all disease groups, chronic biliary disease is highlighted as a particular diagnostic challenge.

Genetic and pediatric liver diseases

P1211
EFFICACY AND SAFETY OF SEBELIPASE ALFA IN CHILDREN AND ADULTS WITH LYSOSOMAL ACID LIPASE DEFICIENCY: RESULTS OF A PHASE 3 TRIAL

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Background and Aims: Lysosomal Acid Lipase (LAL) Deficiency is a progressive multisystem disease that is an underappreciated cause of cirrhosis, severe dyslipidemia and early onset atherosclerosis.
Methods: A phase 3, double-blind, placebo (PBO) controlled trial (NCT01757184) randomized affected children and adults (N = 66) to pbo or sebelipase alfa (SA) 1 mg/kg every other week for 20 weeks. Primary endpoint was ALT normalization. Secondary endpoints included additional important efficacy assessments, safety and immunogenicity. Medically important abnormalities were common at baseline including fibrosis (100%), bridging fibrosis (Ishak score 3 or 4; 47%) and cirrhosis (31%) in biopsied patients (n = 32) and a median LDL-C of 204.0 mg/dL (range 70–378 mg/dl). Mean age of biopsied patients was 12 yr. LDL-C was ≥190 mg/dL in 58% [38 of 66 of patients, including 24% (9 of 38) who were on lipid lowering medications].
Results: After 20 weeks, ALT normalization (ULN range 34–43 U/L) was achieved in 31% of the SA group and 7% of the PBO group. Multiple secondary efficacy endpoints were also met including relative reduction in LDL-C, non-HDL-c, and triglycerides and relative increase in HDL-C. 65 subjects continue in the open label part of the study with data availability to week 36. PBO subjects when transitioned to SA demonstrated effects consistent with the results of the double blind period. Further reductions were observed in those who remained on SA non-HDL-C (−39% vs −28%) and LDL-C (−44% vs −29%) for week 36 versus last double blind visit respectively. Over 350 infusions of SA were given during the double-blind period. The number of patients with AEs was similar in each arm. During the double-blind period, most AEs were mild and unrelated to SA; 6 patients experienced infusion-associated reactions (4 PBO; 2 SA). Dosing was paused in 1 patient after an atypical infusion-related reaction following SA treatment. Safety profile in the open-label period was consistent with the double-blind period. One additional, unrelated SAE was reported (gastroenteritis) in the open-label period.
Conclusions: Sebelipase alfa for 20 weeks demonstrated statistically significant improvements in ALT normalization and in a number of other important disease related abnormalities including marked reductions in LDL. Additionally, open label data show further reductions noted with longer term exposure. The safety profile appears favorable and infusions were generally well tolerated.

P1212
IMPACT OF SEBELIPASE ALFA ON SURVIVAL AND LIVER FUNCTION IN INFANTS WITH RAPIDLY PROGRESSIVE LYSOSOMAL ACID LIPASE DEFICIENCY

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Background and Aims: Lysosomal Acid Lipase Deficiency (LAL D) in infants, historically known as Wolman Disease, is a medical emergency with rapid disease progression and death occurring within the first 6 months (mo) of life. In a natural history study, failure to thrive, liver complications, massive hepatomegaly, and mortality were confirmed in LAL D infants. Disease progression was frequently accompanied by rapidly progressive liver failure. In addition to steatosis and foamy histiocytes, fibrosis was prominent and seen in 4 infants before 6 mo of age and in 1 infant as early as 1.5 mo of age. In infants with evidence of growth failure who did not undergo transplant, the K-M estimate (95% CI) of survival past 1 year of age was 0 (0, 0). Those treated with HSCT or liver transplant survived slightly longer but still died before 1 year of age (median age 8.6 mo).
Methods: A phase 2/3 trial (LAL-CL03) assesses the safety and efficacy of sebelipase alfa (SA) in 9 LAL deficient infants with growth failure in the first 6 mo of life. Median baseline liver function tests reveal significant underlying liver dysfunction with a median ALT and AST of 145 IU/L and 125 IU/L respectively. At symptom onset, subjects had diarrhea/vomiting (n = 6), hepatomegaly (n = 9), splenomegaly (n = 8), or adrenal calcification (n = 6).
Results: As of November 1, 2014, 6 subjects have met the primary endpoint of survival at 12 mo of age with a mean age of 22 mo and 5 continue to receive SA. Deaths (n = 4) were unrelated to SA and were deemed to be either related to underlying disease or, in 1 subject, due to complications of an abdominal paracentesis. 3 subjects died after receiving ≤4 doses. In addition to improved survival relative to the historical cohort, all subjects demonstrated improved weight gain, improvement of GI symptoms, and reductions in hepatosplenomegaly. Rapid improvements in biochemical and hematological markers including ALT, AST, hemoglobin, and bilirubin were observed. One SA-related SAE occurred: an infusion reaction of malaise with tachycardia and fever. The majority of the infusion associated reactions were reported as fever, diarrhea, or vomiting. To date, 4 subjects tested positive to anti-SA antibodies and all 4 continue weekly infusions of SA.
Conclusions: Analysis suggests that SA rapidly improves weight gain and many of the disease activity parameters observed in infants with LAL D. These improvements appear to be accompanied by a substantial survival benefit compared to a matched historical control group.

P1213

ADENO-ASSOCIATED VIRUS VECTOR-MEDIATED LIVER GENE THERAPY FOR CRIGLER–NAJJAR SYNDROME

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Background and Aims: Crigler–Najjar syndrome (CN) is an autosomal recessive rare disorder caused by mutations in the UDP-glucuronosyltransferase 1 isotype A1 (UGT1A1) gene. In severe CN, lack or reduced activity of UGT1A1 results in high levels of serum unconjugated bilirubin (UCB), which can lead to brain damage and death. Treatment of CN consists of phototherapy for 10–12 hours per day, which presents several limitations and has a major impact on the quality of life of patients. Liver transplantation is the only curative option for CN. The limited therapeutic options available prompted us to develop a new therapy for CN based on the transfer of a corrected copy of the UGT1A1 gene to hepatocytes.
Methods: Adeno-associated virus (AAV) vectors are one of the most attractive vectors for in vivo gene transfer. Clinical trial results demonstrate long-term correction of inherited diseases with AAV vectors, particularly for liver-directed gene therapy.
An AAV vector optimized for the liver expression of the UGT1A1 transgene was developed (AAV-UGT1A1). Safety and efficacy of correction of CN with AAV-UGT1A1 were assessed in mouse and rat models of CN.
Results: We demonstrated that a single intravenous administration of AAV-UGT1A1 resulted in efficient targeting of the liver and was sufficient for the long-term correction of CN in affected rats and mice, resulting in UCB levels undistinguishable from wild-type animals. Correction of CN was documented for at least 6 months post-gene transfer (observation ongoing) with no need for immunosuppression or additional interventions.
Conclusions: Long-term correction of the pathological accumulation of UCB in animal models of CN can be achieved at AAV-UGT1A1 vector doses similar to those safely tested in human gene therapy trials. Based on these encouraging results, efforts towards a multicenter clinical trial for AAV-UGT1A1 vector-mediated gene transfer to the liver in severe CN patients have been initiated. Orphan Drug Designation was obtained, and vector manufacturing in GMP and mandatory GLP toxicity studies are ongoing. Screening of CN patients to identify subjects potentially eligible for enrollment in the proposed trial has started in four European countries.

P1214

DIET HIGHLY INFLUENCES THE DEVELOPMENT OF HEPATOCELLULAR ADENOMAS AND CARCINOMAS IN MICE WITH GLYCOGEN STORAGE DISEASE TYPE IA

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Background and Aims: Glycogen storage disease type Ia (GSDIa) is an inherited disorder in which the catalytic enzyme of glucose-6-phosphatase is deficient. GSDIa patients are prone to severe hypoglycaemia and present hepatomegaly and steatosis caused by a large accumulation of glycogen and triglycerides in the liver. In despite of intensive dietary treatment, most patients exhibit hepatocellular adenomas (HCA), which may undergo malignant transformation into hepatocellular carcinoma (HCC) at the third decade of life. Some nutrients such as sucrose, fructose or galactose are restricted or excluded but none specific recommendations about lipid consumption are available. In this study, we propose to determine the effect of junk food on hepatic tumour development in liver-specific G6pc knock-out (L-G6pc$^{-/-}$) mice, which present all hepatic symptoms of the human pathology of GSDIa, including the late development of HCA.
Methods: After the induction of G6pc deletion at adulthood, L-G6pc$^{-/-}$ mice were fed either a STD, or a high fat and high sucrose (HF/HS), or a high fat (HF) diet. After 5, 7 and 9 months of diet, tumour development was followed by in vivo magnetic resonance imaging (MRI). Livers were histologically analysed and liver weight, glycogen and triglycerides stores were measured.
Results: The glycogen and triglycerides storage in livers of L-G6pc$^{-/-}$ mice were not affected by the different diets. Spectacularly, the development of hepatic tumours in L-G6pc$^{-/-}$ mice was highly enhanced on HF/HS and HF diets. Indeed, only 20% of L-G6pc$^{-/-}$ mice have developed millimetric HCA after 9 months of STD diet, whereas first nodules were detected earlier (at 5 months) in 10–30% of L-G6pc$^{-/-}$ mice fed a HF/HS or HF diets, respectively. After 9 months of HF/HS and HF diets, more than 80% of L-G6pc$^{-/-}$ mice developed multiple tumours about 5–15 mm in diameter. Interestingly, about 50% of nodules were proven to be HCC in L-G6pc$^{-/-}$ mice fed a HF/HS diet while most tumours corresponded to HCA in mice fed a STD or HF diet.
Conclusions: These results suggest that tumour development is hugely accelerated with a HF diet. In addition, HF/HS diet induced the malignant transformation of hepatic tumour in mice with GSDIa. Thus the consumption of junk food (fast food) could rapidly damage the liver of patients with GSDIa. Molecular analysis of these tumours would permit us to characterize crucial pathways involved in hepatocarcinogenesis.

P1215

IN VITRO EVALUATION OF ANTISENSE DRUGS IN METABOLIC LIVER DISEASE-SPECIFIC HEPATOCYTE-LIKE CELLS USING IPS-CELL TECHNOLOGY

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Background and Aims: Amyloidoses represent a group of diseases in which a misfolded protein conformation leads to an extracellular tissue deposition, followed by dysfunctions of tissues or organs. Transthyretin (TTR) belongs to this group of about 25 human proteins with amyloid potency that is mainly secreted by the liver. Currently, more than 100 TTR mutations have been discovered in patients with familial amyloidosis (ATTR) with different clinical manifestation. Drug therapy of ATTR is partially possible on a protein level. However, a full silencing of the mutated TTR gene would enormously increase therapeutic outcome. Antisense oligonucleotides (ASOs) and small interfering RNAs (siRNA) are the most commonly used gene-silencing strategies. These artificial nucleotides bind complementary to the target gene mRNA, hence inactivating the translation of the protein. In this study, ATTR patient-specific hepatocyte-like cells (iHeps) were generated from induced pluripotent stem (iPS) cells, followed by a broad *in vitro* analysis of ASOs and siRNA effects on TTR expression.
Methods: Fresh urine (250–500 ml) from ATTR patients was processed for isolation of renal epithelial cells, followed by reprogramming into iPS cells by using integration-free vectors, such as nucleofection of episomal (EBNA) plasmids. After characterization of iPS colonies, a 3-step differentiation protocol toward hepatocytes was performed. Briefly, iPS cells encountered a treatment with specific growth factors (activin A, Wnt3a, FGF2,

HGF) for 14 days. iHeps were then characterized by analysis of typical hepatic markers, e.g. albumin and TTR via RT-PCR, immunocytochemistry and hepatocyte-specific functional assays. For TTR gene silencing, ASOs or siRNAs were introduced into iHeps using cationic lipids with or without electroporation. The phenotype of TTR knockdowns in iHeps was examined *in vitro*.
Results: After 2 weeks of cultivation of urinary cells, 4–6 stable cell populations emerged. Episomal vectors yielded iPSCs with characteristic features and normal karyotype. The iHeps indicated a high similarity to primary human hepatocytes. ASOs or siRNA treatment generated TTR depleted ATTR iHeps.
Conclusions: Urine cell-derived iPSCs can be reprogrammed and efficiently differentiated to iHeps. Antisense drugs are suitable tools for TTR knockout in ATTR iHeps. As a result, ASOs or siRNA could be potentially useful for pharmacological development and regenerative medicine.

P1216
ADVANCED LIVER DISEASE CORRELATES WITH LOW SERUM LYSOSOMAL ACID LIPASE ACTIVITY

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Background and Aims: Deficiency of lysosomal acid lipase (LAL) causes Wolman disease (WD) and cholesteryl ester storage disease (CESD) due to organ infiltration by macrophages filled with cholesteryl esters and triglycerides. Decreased LAL in various degrees may be asymptomatic, cause fatty liver or even cirrhosis. Replacement enzyme therapy is currently available in clinical trialsto study LAL levels in patients with Non Alcoholic Fatty Liver Disease (NAFLD) and idiopathic cirrhosis.
Methods: Medical records of patients aged 1 year to 65 years that underwent liver biopsy from the years 2006 to 2012 were screened. Patients with biopsies showing cryptogenic cirrhosis, microvesicular steatosis or metabolic syndrome related macrovesicular steatosis, were screened for LAL activity using the new method of dried blood spots specific assay (Synageva Inc.). Clinical, laboratory, imaging and pathological data was collected and analyzed.
Results: Of those patients screened 4 had cryptogenic cirrhosis, 9 had microvesicular steatosis and 9 had macrovesicular steatosis. The mean age was 32.4±23.3 (ranges 2.9–71.8) years. Mean LAL activity was 0.74 (median 0.8, ±0.28) nmol/punch/hour. Three cases had LAL <0.37 (carriers) but none of the 22 cases had LAL <0.03 (diagnostic for WD & CESD). However, threshold of LAL activity <0.6 (as compared to 0.4 and 0.5), in seven of the 22 cases allocated severity markers of liver disease: low serum calcium, hypoalbuminemia, low total protein and prolonged INR due to synthetic dysfunction, low platelets due to hypersplenism and portal hypertension, high serum uric acid a result of low blood volume, and impaired glucose and HbA1c levels suggesting insulin resistance.
Conclusions: LAL activity <0.6 is associated with severe liver injury in patients with fatty liver and cirrhosis. Although our pilot study is small and had biases the results are in-line with other reports suggesting lysosomal alterations in NAFLD. Further larger studies are warranted to define the relation of LAL levels to the severity of liver disease and the need for replacement therapy in those cases.

P1217
DYNAMICS OF HUMAN LIVER PROGENITOR CELLS IN THE PERINATAL PERIOD

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Background and Aims: In humans, insufficiency of hepatocytes to contribute adequately to liver regeneration will activate the normally quiescent liver progenitor cells (LPC) which is recognizable as ductular reaction. Results of several studies suggest parallels between fetal development and adult liver regeneration but little is known about LPC behaviour in the human liver in the perinatal period. We investigated the patterns of LPC in livers of children who died after 28 weeks of gestation, either in utero or within 1-week postpartum.
Methods: 52 cases were selected based on absence of liver diseases. All samples were stained with CK7 to highlight the LPC. Portal CK7+ cells were scored as number of quadrants per portal tract (PT) in which CK7+ cells are present (1 to 4) and 10 PT per sample were scored. Strings of small parenchymal CK7+ cells, phenotypically matching LPC were counted as LPC-complexes (LPCcx) in 10 hotspots per sample. CK7+ intermediate hepatobiliary cells (IHBC) were counted separately in 10 hotspots per sample.
Results: Based on the staining pattern of the portal CK7+ cells, cases can be divided in 2 groups: one with a ductal plate pattern (DP, n = 14) and a group showing mature PT (MPT, n = 38) containing ductular reaction. There were no significant differences in gestational age, clinical condition and time of death between the 2 groups. Also no differences in LPCcx and IHBC presence was seen, suggesting that there is a variable pattern of PT maturation regardless of gestational age.
We subdivided the cases in those with and without IHBC to assess parenchymal regeneration. In the DP group a borderline difference in LPCcx was found ($p = 0.06$) showing higher numbers of LPCcx in the IHBC positive group. In the MPT group a higher number of LPCcx was seen in the IHBC positive cases ($p = 0.04$) whereas no difference was seen in the scoring of ductular reaction. Increase of LPCcx parallels the presence of IHBC regardless the PT pattern.
Conclusions: In perinatal livers there is a variable pattern of PT maturation unrelated to gestational age. Regardless the PT maturation pattern, parenchymal LPC activation can be present indicating parenchymal regeneration. The latter does not parallel the portal regenerative activity expressed as ductular reaction.

P1218
ALPHA-1 ANTITRYPSIN DEFICIENCY AND LIVER DISEASE IN CHILDHOOD

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Background and Aims: Alpha-1 antitrypsin deficiency (A1ATD) is a genetic disease, relatively common in individuals of Northern European and Iberian descent. The most common abnormal alleles are Z and S, and majority of individuals with severe A1ATD have ZZ phenotype (PiZZ). It is known that about 10% of affected infants will develop some form of liver disease. After biliary atresia (BA), A1ATD is the second cause of liver transplantation (LT) in childhood, in our country. We aim to characterize pediatric population with A1ATD and liver disease followed in the liver unit, and those who were referred for LT, of a tertiary pediatric hospital.
Methods: A retrospective review of the medical records of patients diagnosed with A1ATD was made (December 1994 to October 2014). Demographic, clinical and laboratorial parameters were studied. Two groups were considered: those who underwent LT and those

who did not. Statistical analysis used *SPSS*® 18.0 ($p < 0.05$ statistically significant).
Results: The study included 41 patients, 24 (58.5%) boys. Median age at diagnosis was 2 months (15 days to 16.8 years; 1 with prenatal diagnosis). Median follow up was 7.9 years. Majority (82.9%) of patients were from northern and central regions of our country. There was 33 PiZZ, 5 PiSZ, 1 PiSS, 1 PiMZ and 1 PiMS. Enlarged liver and/or spleen (29) and neonatal cholestasis (28) were the most common clinical presentations. Five patients had intrauterine growth restriction (2 premature), 12 had failure to thrive at presentation. From the 21 patients who underwent liver biopsy, 13 had abnormalities consistent with A1ATD. Regarding comorbidities, 4 patients had asthma (1 PiZZ, 1 PiSZ, 1 PiMZ, 1 PiMS), 4 had BA (3 PiSZ, 1 PiZZ), and 1 had mitochondrial disease (PiZZ). Twenty-five patients (61%) progressed to end-stage liver disease and underwent LT (23 PiZZ, 2 PiSZ with BA) and 2 required retransplant. Median age at first LT was 4.3 years. Infection (25%) was the most common complication after LT. Statistical significance ($p = 0.016$) was found for AST level at presentation (mean AST in LT group 227 U/L *versus* no LT group 129 U/L). Three patients died, 1 following LT (primary non function), 2 due to comorbidities (primary immunodeficiency; mitochondrial disease).
Conclusions: It is highlighted that other severe diseases involving liver could be present. A1ATD can be a severe disease as nearly two thirds of the patients had progressed to end-stage liver disease requiring LT. AST level at presentation was shown to be a marker of disease severity.

P1219
HEPATIC HEDGEHOG SIGNALING AND STEROIDOGENESIS – AN EVIL PARTNERSHIP?
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Background and Aims: The liver has multiple anabolic and catabolic functions. Novel results showed that Hedgehog (Hh) signaling, a pathway commonly associated with embryogenesis, development and cancer, is active in hepatocytes and acts as a master regulator of zonation in the adult liver (Gebhardt and Matz-Soja, WJG, 2014). We are interested in how this signaling pathway is connected to sex-specific regulation of gene expression.
Methods: For these investigations we bred a transgenic mouse with an inactivated Hh signaling, by a hepatocyte-specific knockout (KO) of Smoothened, a key protein of Hh signaling (SAC mice). Homozygous female SAC KO mice are unable to reproduce whereas syngeneic male littermates were fertile. To explain these observations we performed histological and molecular biological analyses of liver and ovary.
Results: The results showed an infantile reproductive system and no histological features of oestrus cycle activity. These manifestations were associated with alterations in the gene expressions of hepatic insulin like growth factor 1 and binding protein 1 (*Igf1, Igfbp1*; Matz-Soja et al., CCS, 2014). Surprisingly we observed alterations in steroidogenic gene expression as well; yet it was assumed that steroidogenesis occurs in the liver only during embryogenesis and is down-regulated afterwards. We found changes in expression of several steroidogenesis associated genes, like cytochromes (e.g. *Cyp17a1, Cyp11A1*, *Cyp19a1*) and hydroxysteroid dehydrogenases (*Hsd3b1*, *Hsd17b2*). In SAC KO mice the expression of *Cyp17a1* was dramatically increased in both genders relative to wild type mice with an even higher expression in males. No such changes were found in the ovaries. Furthermore we observed an up-regulation of estrogen receptor (*Esr1*) gene expression, relevant for steroid hormone mediated signaling and a clear correlation to the also up-regulated *Cyp17a1* expression. Most of these changes could likewise be induced by transfection of cultured hepatocytes with Gli3 siRNA.
Conclusions: Collectively, the experiments showed a clear influence of the morphogenic Hh pathway on the reproductive system and the regulation of steroidogenesis. Apparently, these effects reflect just an example of the general effect of this morphogenic pathway on gene expression rather than an altered hepatic steroidogenesis feed-back through different levels of sex hormones. These unexpected findings are promising for future studies to improve our understanding of the gender dimorphism of regulation in liver.

P1220
INTERACTION OF TLR-IFN AND HLA POLYMORPHISMS ON SUSCEPTIBILITY OF CHRONIC HBV INFECTION IN SOUTHWEST HAN CHINESE
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Background and Aims: The innate immunity represented by TLR-IFN signal pathway plays a crucial role in HBV infection. HLA polymorphisms are associated with chronic HBV infection. We aimed to explore TLR-IFN gene polymorphisms association with chronic HBV infection and interaction between TLR-IFN and HLA gene polymorphisms.
Methods: In Chinese Southwest Han population, 1191 chronic HBV infection and 273 HBV clearance were selected as case and control, respectively. A total 39 SNP loci for 23 TLR-IFN gene (TLR3 rs3775296, TLR4 rs11536889 and rs1927907, TLR5 rs5744174, TLR9 rs352140, MYD88 rs7744, IRAK1 rs1059702 and rs1059703, IFNG rs2069705, IFNGR1 rs3799488 and rs10457655, IFNGR2 rs1059293 and rs2834211, IL15 rs10519613 and rs10833, IL28B rs12979860 and rs8099917, IL29 rs30461, IL6 rs1800796, EBI3 rs4905 and rs6613, IL12B rs3212227, NFKB1 rs4648068, NFKB2 rs7897947, IL1B rs1143623, rs1143627, and rs16944, IL2 rs2069772, IL10 rs1800872 and rs1800896, IL12A rs2243115 and rs568408, CXCL10 rs4256246, rs4508917, and rs8878, MX1 rs1557370, rs2070229, rs467960, and rs469390) were selected for genotyping. Four HLA SNP loci associated with chronic HBV infection by GWAS, rs3077, rs2856718, rs9277535, and rs7453920 were selected to explore the gene-gene interaction between HLA and TNF-IFN. SNPStats and MDR were used for statistical analysis.
Results: Significant association was seen in rs352140 (OR = 0.70, p = 0.0088), rs16944 (OR = 0.67, p = 0.016), rs3212227 (OR = 1.38, p = 0.021), rs3799488 (OR = 1.48, p = 0.0048), rs1059293 (OR = 0.27, p = 0.011), rs467960 (OR = 0.68, p = 0.022), as well as four SNP loci in HLA. A synergistic relationship was seen between rs9277535 and rs16944 (0.13%), rs1143623 and rs6613 (0.10%). The combination of rs9277535 and rs16944 was the best model to predict chronic HBV infection (TA = 0.6040, p = 0.0010, CVC = 10/10).
Conclusions: TLR-IFN gene polymorphisms are associated with chronic HBV infection. Interacted with TLR-IFN polymorphisms may be one mechanism for HLA polymorphism to influence the susceptibility of chronic HBV infection. The combination of rs9277535 and rs16944 is better than rs9277535 to predict chronic HBV infection.

P1221

IL12A GENE POLYMORPHISM IS CLOSELY CORRELATED WITH HBeAg SEROCONVERSION IN ENTECAVIR TREATED CHRONIC HEPATITIS B PATIENTS

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Background and Aims: Host genetic factor is one of important reasons for different response of antiviral treatment in chronic hepatitis B. IL-12A play an important role in immune to HBV. IL12A rs568408 is association with many diseases. The study aimed to observe the association between IL12A gene polymorphism and HBeAg seroconversion in chronic hepatitis B patients who received entecavir treatment.
Methods: IL12A rs568408 was genotyped using the MGB-TaqMan SNP genotyping assay in 109 HBeAg-positive chronic hepatitis B patients who had received entecavir treatment. After 24 months, 29 cases achieved HBeAg seroconversion (response) were assigned as the case and the remaining 80 cases were assigned as the control. SNPstats was applied to analyze the association between IL12A rs568408 and HBeAg seroconversion.
Results: IL-12A rs568408 was associated with HBeAg seroconversion (OR 3.72, 95% CI 1.34–10.32, p = 0.012). GA genotype achieved higher HBeAg seroconversion rate than GG genotype.
Conclusions: IL-12A rs568408 was significantly associated with HBeAg seroconversion in HBeAg-positive chronic hepatitis B patients who received entecavir treatment.

P1222

CIRRHOSIS AND CARCINOMA IN COURSE OF CHRONIC HEPATITIS C IN CHILDREN

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Background and Aims: Chronic hepatitis C (CHC) can lead to cirrhosis, cancer and finally hepatic failure. These serious complications are observed after years of HCV infection, and therefore by far more frequently in adults patients than in children. Retrospective analysis of the frequency of serious complications: cirrhosis and cancer in course of CHC in children, as well as clinical characteristic of patients with these complications.
Methods: The research included 146 children with CHC who were subject to treatment in our Department from 1991 to 2013. The diagnosis of CHC was established based on clinical symptoms, the detection of HCV antibodies (enzyme immunoassay), and/or HCV RNA (RT-PCR method), and by histological changes, grading the activity (G0–G4) and histological progression – staging (F0–F4) in the liver.
Results: Cirrhosis (F3/F4) was diagnosed in 7 children (4.79%) with CHC caused by genotype 1 of HCV (1a and 1 b in 4 and 3 patients respectively). All these children were infected during hospitalization, 6 of them under previous oncological therapy. Symptoms of hepatic failure were observed in 3 children (2.05% of whole group, 42.85% of children with cirrhosis). Cancer was diagnosed in 1 patient, ten years old boy with Down syndrome and cirrhosis in course CHC, caused by genotype 1b of HCV. Among symptoms of cirrhosis were observed: edema, ascites, splenomegaly, signs of portal hypertension, coagulation defects, weakness, asitia, abdominal pain, icterus, ginecomastia. In 4 children with cirrhosis coexistence of others chronic illnesses were found: in 2 children Down syndrome, in 1 common variable immunodeficiency, in 1 coeliac disease.
Conclusions: The parameter which increases the risk of cirrhosis and hepatic failure in course of CHC in children is congenital immunodeficiency including Down syndrome.

P1223

FRAILTY IN CHILDREN: A NOVEL TOOL THAT MEASURES THE MORBIDITY ASSOCIATED WITH END-STAGE LIVER DISEASE

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Background and Aims: Frailty is a syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines and causing vulnerability to adverse outcomes. Frailty (composed of 5 classic Fried criteria) can be reliably and reproducibly measured in adults and is a powerful predictor of mortality. A large study of adults listed for liver transplantation (LT) demonstrated that 17% were frail and that frailty scores strongly predicted wait list mortality. To-date, frailty has not been investigated in children with liver disease. We hypothesized that (i) frailty can be measured in children; and (ii) frailty assessments can identify morbidity in children with end-stage liver disease that is not captured by available scoring systems.
Methods: This was a prospective multi-center study of children (5–17 years) with chronic liver disease listed for LT (Listed) or with chronic liver disease without evidence of decompensation (Control). Fried frailty elements were assessed with either the same adult tools (i) weakness (grip strength with hand-held dynamometer) & (ii) slowness (6-minute walk distance) or with validated paediatric tools (iii) shrinkage (triceps skinfold thickness), (iv) exhaustion (PedsQL™ Multidimensional Fatigue Scale) & (v) physical activity (modified Physical Activity Questionnaire). In adults each element is scored as abnormal (1) or normal (0) leading to a 5-point scale with 5 representing the most frail patients. This was adapted in children to abnormal (2), suboptimal (1) and normal (0) resulting in a 10-point scale. Each score was assigned based on standard deviations (sd) of the mean in validated norms (2: <2sd, 1: ≥−2sd to ≤−1sd, 0: >−1sd).
Results: 46 frailty assessments were completed in 14 Listed and 32 Control subjects at 12 sites. Median age was 11.9 yrs. Median time to complete the assessments was 76.9 min in Listed and 58.4 min in Controls. Using a cut-off of 6/10 defining frailty (comparable to a validated threshold of 3/5 in adults), 7/14 (50%) Listed children were frail compared to 0/32 Controls (p < 0.0001). There was no association between frailty and MELD/PELD scores.
Conclusions: Frailty assessments in children are feasible. In our cohort, frailty was present in 50% of children listed for LT. Frailty and MELD/PELD scores are independent and therefore frailty captures an under-appreciated aspect of morbidity in end-stage liver disease. Frailty assessments may be useful to optimize the selection and timing of paediatric LT.

P1224

NEXT-GENERATION SEQUENCING FOR THE DIAGNOSIS OF WILSON'S DISEASE

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Background and Aims: Wilson's disease (WD) is an autosomal recessive disorder of copper metabolism with ATP7B gene mutation at chromosome 13 in the background. More than 550 disease-causing mutations have been identified. The appearance of the next-generation sequencing (NGS) opened a new era making possible quick, one-step detection of all mutations.
Methods: Ion Torrent Personal Genome Machine was used for detecting ATP7B mutations in clinically diagnosed Wilson patients with 4 or higher score according to the international diagnostic system. 3 known homozygous, 5 compound heterozygous patients with only one known mutation and 1 patient without any known mutation were involved. The mutations detected by NGS were validated by the traditional Sanger sequencing.
Results: The average time of sequencing was 8 hours. In each 9 patients (7–51 years; 4 with liver disease, 1 with only neurological symptoms and 3 with both hepatic and neurological manifestations and 1 symptomless sibling) the mutations on both alleles have been identified strengthening the Wilson's disease diagnosis. All the formerly known mutations have been confirmed by NGS and the other mutations have been identified in the heterozygous patients. One missense mutation (R969Q) have been detected in 2 non family member patients, 5 other mutations (M769L, A1063V, P1134P-fs, L1305P and Q1351X) were identified in single patients. The H1069Q mutation was present in 8 out of 9 patients (3 homozygous and 5 heterozygous).
Conclusions: Although the international score system helps to set-up the diagnosis of WD, in some cases it still remains a challenge. The next-generation sequencing is a fast and relatively cheap method for detecting the wide spectrum of disease-causing mutations of ATP7B gene.

P1225

LIVER STIFFNESS MEASUREMENT IN PATIENTS WITH WILSON DISEASE

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Background and Aims: Liver stiffness measurement (LSM) using transient elastography (TE) is non-invasive method for fibrosis assessment in chronic liver diseases. Its performance in patients with Wilson disease (WD) is incompletely specified yet. The aim of our study was to evaluate the LSM in WD patients.
Methods: Liver stiffness in 38 WD patients was measured by FibroScan®402 (Echosens, Paris, France, M probe). We compared the sonographic evaluation of the normal and reduced elasticity. In 13 patients, liver biopsy was available for comparison.
Results: The patients with liver disease only (n = 17) had a mean value of 12 kPa. The patients with a mixed WD form (n = 17) presented with a mean value of 16.8 kPa. Purely neurological patients (n = 2) had a value of 6.15 kPa while two presymptomatic individuals had a value of 4.9 kPa. Ten patients had normal elasticity (mean of 5.21 kPa, F0). Sonographic assessment of slightly reduced liver elasticity corresponded to the mean value of 7.77 kPa. The patients with moderately reduced elasticity received a mean value of 11 kPa. Rigid liver coincided with a high value of 29.9 kPa (F4). Ultrasound evaluation of advanced fibrosis corresponded to the value ≥11 kPa (F3) in 17 patients. Twelve of them were with liver cirrhosis and with a mean value of 45.39 kPa (11.5–69.1 kPa, F4). Seven patients had histologically proved fibrosis. There was liver stiffness of F3–F4 in four of them while the other three presented with F0–F1. Six individuals with liver biopsy had no fibrosis at all. All the patients except one were with F0 Fibroscan score.
Conclusions: TE is non-invasive, rapid and helpful method for LSM. More investigations are needed to validate LSM in WD. Our experience shows that Fibroscan assessment correlates with clinical and ultrasound evaluation of the liver stiffness. Our preliminary results indicate that it is a reliable diagnostic tool in clinical practice when managing WD patients.

P1226

SERUM BILE ACIDS IN NEONATES: DETERMINATION OF NORMAL VALUES IN FED AND FASTENED CONDITION

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Background and Aims: Serum bile acids (BA) are high in neonates and gradually fall, reaching adult values by age 11 (Jahnel et al. 2014, unpublished data). BA are mostly conjugated to glycine; however – since taurine is abundant in breast milk and supplemented in infant formulas – in neonates BA are mostly conjugated to taurine. We aimed to measure BA composition in healthy neonates to determine standard values for this age group.
Methods: Serum total BA (tBA) concentrations were measured in 16 healthy term neonates without cholestasis on the third day after birth. Neonates were divided into two groups: Fasted (n = 5) and fed (n = 11). Using high-performance liquid chromatography–high-resolution mass spectrometry to analyse 10 µl of serum, we created 15-BA profiles including unconjugated and taurine- or glycine-conjugated BA.
Results: In fed neonates tBA values (16.3±5.8 µmol/l) were higher than in fasted neonates (5.4±3.3 µmol/l). In fed vs. fasted neonates taurine-conjugated BA (10.2±2.8 µmol/l vs. 2.3±0.6 µmol/l) were clearly more abundant than glycine-conjugated BA (5.8±0.5 µmol/l vs. 2.1±0.5 µmol/l). Primary taurocholic acid, taurochenodeoxycholic acid, and glycocholic acid were the most abundant BA, whereas levels of all secondary BA were <0.1 µmol/l. No differences in tBA profiles were visible between breast-milk-fed and formula-fed neonates.
Conclusions: This study confirms that taurine-conjugated BA are the predominant BA species in neonates. Since BA may serve as non-invasive biomarkers in hepatic and gastrointestinal disorders, to determine age-specific normal fed and fasted BA value ranges in neonates is essential.

P1227

INTRA-HEPATIC CHOLESTASIS OF PREGNANCY (ICP): PREVENTION OF RECURRENCE OF ICP AND INTRA-UTERINE FETAL DEATH BY PRE-PRURITUS ADMINISTRATION OF URSODEOXYCHOLIC ACID

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Background and Aims: Ursodeoxycholic acid (UDCA) is a recognized efficient treatment of established generalized pruritus (GP) and biochemical abnormalities of intra-hepatic cholestasis of pregnancy (ICP), a disorder associated with possible fetal death

and a 70% rate of recurrence in the following pregnancies. However, UDCA is not always effective and whether it may obviate recurrence of ICP and in utero fetal death has not been demonstrated. The aim of this study was to show that, in some women who sustained ICP at their initial pregnancy, early administration of UDCA during a further pregnancy may prevent the recurrence of ICP and also in utero fetal death.

Methods: Pregnant women with a previous history of ICP agreed to ingest UDCA in the absence of pruritus in the 2nd trimester of a new singleton pregnancy. The daily dose of UDCA ranged between 12 and 16 mg/kg/day up to delivery.

Results: Four women (22–31 year-old) developed GP and abnormal liver blood tests between the 26th and 36th week (wk) of their 1st singleton pregnancy. In one woman with a familial history of symptomatic gallstones, UDCA was not administered during her 1st pregnancy (associated with *in utero* fetal death at 33 wks of gestation) and during her 2nd (twin) pregnancy (associated with GP from the 18th wk of gestation and death of the twin neonates after 5 days following delivery at the 24th wk of gestation). During the 3rd and 4th pregnancies, UDCA (15 mg/kg/day) was given before the occurrence of pruritus, from the 15th and the 17th wks and up to the end of gestation. Both pregnancies were not complicated by pruritus and ended by delivery of normal neonates at 35 wks of gestation. In the 1st pregnancy of the 3 other women, pruritus occurred for 10 days to 4 wks, 2 women were given low dose of UDCA for less than 4 wks successfully, and all were delivered at 35–36 wk of gestation (one cesarean delivery; one low-weight neonate with respiratory distress). The all 3 women had another singleton pregnancy during which UDCA was given before the occurrence of pruritus, between 17 and 24 wks of gestation; pruritus was not observed, normal delivery took place at 35–37 weeks of gestation and the 3 neonates were normal.

Conclusions: in some women with a history of ICP, pre-pruritus administration of UDCA from the 2nd trimester to the end of a further pregnancy may prevent the recurrence of ICP and, even, intra-uterine fetal death.

P1228

CHANGED SERUM BILE ACID LEVELS IN CHILDREN AND ADOLESCENTS WITH ACUTE INFECTIOUS ENTERITIS OR INFLAMMATORY BOWEL DISEASE

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Background and Aims: Bile acids (BA) undergo highly efficient enterohepatic circulation which is impaired in acute infectious diarrhea (AID) and in inflammatory bowel disease (IBD) due to disturbed reabsorption of BA in the terminal ileum and colon. Decreased portal-venous concentrations of BA lead to an upregulation of *de novo* BA synthesis in hepatocytes, resulting in increased peripheral-blood BA levels. We aimed to quantitate the impact of inflammation of the gastrointestinal tract on BA metabolism.

Methods: Serum BA levels were measured in patients with AID (n = 57) due to viral infection or in IBD patients with active disease (n = 28; 85 overall, ages 0–18 years). Patients with IBD were further divided into two groups: ulcerative colitis (n = 15) with acute colitis and Crohn's disease with terminal ileitis (n = 13). Healthy children and adolescents (n = 197) served as controls. IBD patients with primary sclerosing cholangitis served as positive controls. 10 µl of serum was required for creating a 15-BA profile including unconjugated and taurine- or glycine-conjugated BA by high performance liquid chromatography–tandem-mass spectrometry.

Results: In the AID group BA levels were significantly decreased (2.9±3.8 µmol/L) compared to normal ranges (4.3–6.4 µmol/L; $p < 0.01$). In IBD patients BA levels were significantly decreased in Crohn's disease patients (3.1±1.9 µmol/L; $p < 0.01$ vs. controls) as well as in the ulcerative colitis group (3.2±2.0 µmol/L; $p < 0.01$). Glycine- and taurine-conjugated BA were both elevated in this group, especially glyco-ursodeoxycholic acid Interestingly, patients suffering from IBD and primary sclerosing cholangitis (n = 5) had highly elevated serum BA levels (95.8±89.8 µmol/L).

Conclusions: This study shows that an acute viral enteritis as well as chronic intestinal inflammation impairs BA re-uptake leading to significantly reduced serum BA levels. Whether medical manipulation of the intestinal BA transport in IBD or acute enteritis favorably affects symptoms like diarrhea requires further investigation.

P1229

LIVER TRANSPLANTATION FOR PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS TYPE 3 (MDR3 DISEASE) PRESENTING IN THE 5TH DECADE OF LIFE

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Background and Aims: PFIC a rare heterogeneous group of autosomal-recessive disorders that presents during the neonatal period or within the first year of life resulting in intra-hepatic cholestasis during childhood. PFIC3 generally occurs later presenting either in late infancy, childhood, or even early adulthood [The Multiple Facets of ABCB4 (MDR3) Deficiency].

Case report: 44-year old gentleman with history of DM and jaundice since childhood due to Dubin–Johnson syndrome without itching, he developed progressive jaundice and intractable itching associated with dark urine and pale stools, over the past 2 years, his total bilirubin was 563umol/L with high GGT and very high serum bile acid. Serology for viral hepatitis, autoimmune hepatitis, Wilson, alpha 1 antitrypsin and hemochromatosis were negative. Abdominal US showed no bile duct dilatation. MRCP showed Moderate splenomegaly without evidence of primary sclerosing cholangitis (PSC), liver biopsy showed Chronic liver disease (stage II/IV) with finding suggestive of small duct PSC, and Dubin-Johnson syndrome.

He was started on hemodialysis for biopsy proven diabetic nephropathy 1 year ago.

Results: Progressive familial intrahepatic cholestasis (PFIC3) was considered and liver biopsy stained negative for MDR3 by immunostaining and total absence of MRP2 (Dubin Johnson protein), and genetic testing revealed a combination of Dubin-Johnson mutations and MDR3 mutations. He received Living related liver transplantation from his daughter on September 16, 2014 and he has an excellent graft function, awaiting for kidney transplant.

Conclusions: PFIC 3 may present in adults leading to progressive liver disease requiring liver transplantation.

P1230

CHANGED SERUM BILE ACID LEVELS IN CYSTIC FIBROSIS

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Background and Aims: Cystic fibrosis (CF) may affect the liver. We investigated changes in serum bile acid (BA) levels in different ages in CF patients with the aim to evaluate BA as a serum marker for CF associated liver disease.

Methods: The concentrations of BA were measured by the liquid-chromatography-tandem mass spectrometry in patients suffering from CF, divided into 3 age groups and in groups with and without ursodeoxycholic acid (UDCA) treatment. BA levels were correlated

with laboratory values like bilirubin, the alkaline phosphatase activity (AP), and elevated gamma-glutamyl transferase activity (GGT), and serum transaminase activity (AST, ALT).
Results: BA levels were measured in 49 patients. Most patients had continually UDCA-treatment (n = 36) and showed BA values significantly above the reference ranges: <11 years: 11.7±6.2 μmol/l (3.61–6.41 μmol/l, p < 0.01; n = 7), 12–19 years: 6.3±7.8 (3.09–4.12; p < 0.01; n = 15), >19 years: 7.9±4 (3.09–4.12; n = 14). The group without UDCA treatment also showed higher serum BA concentrations without reaching significant values: <11 years: 5.1±4.8 (n = 2), 12–19 years: 5.77±0.96 (n = 3) and >19 years: 4.4±4 (n = 8). No group had significant changes of routine liver serum parameters (AP, GGT, AST and ALT levels) compared to healthy controls; however the average values of AST, ALT and GGT in the group without UDCA treatment were higher than the average values in the group with UDCA treatment.
Conclusions: BA values are mostly increased in CF patients due to UDCA treatment. BA in serum seems not to be early marker for progredient liver disease in CF, sonography the liver seems to be indispensable. However, BA measurements could be used to test the compliance of the CF patients concerning regularly UDCA intake.

P1231
POTENTIAL OF INDUCED PLURIPOTENT STEM CELLS FOR THE TREATMENT OF CRIGLER–NAJJAR LIVER DISEASE

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Background and Aims: Combination of gene therapy and hepatocyte (HEP) transplantation is a promising alternative to liver transplantation for many inherited metabolic liver diseases, such as Crigler–Najjar (CNI). Nevertheless, the major obstacle that remains is the insufficient amount of corrected HEP available for transplantation. Induced pluripotent stem cells (iPS) are endowed with intrinsic self-renewal ability and the potential to differentiate into any of the three germ layers, allowing cell amplification before differentiation. As such, iPS are heralded as the most promising avenue for cell-based therapeutics. However, recent evidence indicates that genetic and epigenetic features of iPS are heterogeneous and could affect the safety of the approach.
Methods: Several iPS clones from CNI patient-specific HEP were generated and efficiently differentiated to HEP-like cells (HLC). In order to show the potential of these cells for gene therapy, CNI-HLC were transduced with a lentiviral vector expressing the UDPGT protein, missing in CNI disease, under the control of a hepatic promoter. Then, transcriptomes of parental CNI-HEP, CNI-iPS and CNI-HLC at different time-points were analyzed by RNA-sequencing.
Results: The heat-map revealed sample clustering according to their potency level. Interestingly, many differences appeared between CNI-HEP and CNI-HLC, with higher expression of many transcripts in the latter. Also, many endogenous retroelements, which are known targets of epigenetic silencing upon embryonic stem cell differentation, were induced by the reprogramming of the CNI-HEP to iPSC. However, most returned to basal levels upon differentiation to CNI-HLC, indicating that this phenomenon was largely reversible.
Conclusions: Hepatocyte-derived iPS cells display epigenetic anomalies translating in the upregulation of many endogenous retroelements. While expression of these elements seemed largely suppressed upon ex vivo differentiation of these iPS into HLC, our results warrant further examination of the epigenetic features of iPS-derived hepatocytes before these can be considered for clinical application.

P1232
FUNCTIONAL CHARACTERIZATION AND DRUG RESPONSE TO ZINC AND D-PENICILLAMINE OF DISEASE-CAUSING ATP7B MUTATIONS IN A HUMAN HEPATOMA CELL LINE

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Background and Aims: In Wilson disease (WD) hepatocyte survival is significantly affected by toxic copper (Cu) overload due to mutation of the liver copper transporter ATP7B. More than 600 mutations of ATP7B are described. Copper toxicity in WD is reversed commonly by D-penicillamine (DPA) and/or zinc (Zn). The impact of the individual ATP7B mutation expressed in WD patients for efficacy of standard treatment is not known.
Methods: ATP7B mutations that are frequent in Europe/USA and Asia as well as various frequent homozygous genotypes observed in India were selected. Stable cell lines were established expressing the mutations in a human hepatoma HepG2 ATP7B knockout cell line that was established recently by our group (PLoS ONE, 9: e98809, 2014). Cell survival as well as induction of apoptosis was determined in mutant cell lines following Cu exposure and Zn/DPA treatment.
Results: In the mutant cell lines ATP7B protein expression varied while mRNA expression was on similar levels as compared to wild type. Co-localization studies with late endosome-lysosome marker lamp2 suggested impairment of trafficking in various mutant cell lines. Mutant cell lines showed different grades of activity to escape toxic Cu with mutation p.H1069Q displaying a moderate activity. No activity was observed for mutant cell lines encoding incomplete reading frames. Characteristic responses to treatment were observed after Zn, DPA and Zn+DPA combined treatment for individual cell lines. Overall, DPA treatment was more efficient as compared to Zn treatment. Of note, only Zn+DPA treatment could fully restore ATP7B activity as compared to ATP7B wild type.
Conclusions: Individual ATP7B mutations differ in their capacity to escape toxic Cu and show genotype-specific response to treatment. The type of ATP7B mutation may modulate the efficacy of Zn and DPA treatment suggesting that current regimens might further improve using the knowledge of functional parameters exerted by ATP7B.

P1233
RETARGETING OF BILE SALT EXPORT PUMP (BSEP) AND CRITERIA OF FAVOURABLE OUTCOME IN CHILDREN WITH PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS TYPE II (PFIC-II)

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Background and Aims: To investigate predictors of clinical evolution in PFIC-II patients and their relationship to BSEP expression and (re)targeting.
Methods: 23 children with established PFIC-II were retrospectively included. Clinical, biochemical and histological characteristics were reviewed at presentation and following treatment with Ursodeoxycholic acid (UCDA) only (10 mg/kg TDS) (n = 20) or UCDA and Partial Biliary Diversion (PBD) (n = 3). BSEP immunostaining was obtained in 20/23 patients. Response to treatment was defined as normalization of pruritus, disappearance of jaundice, and normalisation of alanine amino transferases (ALT) (<1.5 upper limit of normal – ULN). The duration of remission was also recorded.

Six responders had a paired biopsy with BSEP immunostaining, 2 after PBD.

Results: Twelve of 23 patients were non-responders, 1 partial responder and 10 responded to treatment. Non-responders had earlier onset of jaundice (<9 months), neonatal cholestasis and higher ALT levels. ALT >165 IU/L had sensitivity of 72% and specificity of 55% to predict non-response.

Eight of 12 non-responders had no BSEP expression, 1 cytoplasmic, 1 canalicular, 2 not done. Amongst 10 responders, 5 had cytoplasmic BSEP expression and 5 absent. Paired biopsies were obtained after treatment in 6/10: De novo canalicular expression of BSEP occurred in 4/6, 2 with baseline cytoplasmic expression and 2 with no baseline expression.

Seven patients were still responders at last follow up (median 20 months, range 5–67 months), one of them died from unrelated cause, and 3 did relapse after 56, 72 and 82 months. Amongst the 9 living responders a median relapse free survival time of 72 months (95% CI 48 to 96 months) was observed and the five years Kaplan–Meier relapse free survival was 75% (95% CI 33–100%).

Conclusions: PFICII children with late onset presentation, ALT <165 IU/L and cytoplasmic BSEP are likely to respond at least transiently to non-transplant treatment while patients with neonatal cholestasis do not. All but one PFIC II patients had abnormal or no BSEP expression. De novo or retargeted canalicular expression of BSEP can occur under treatment amongst the responders.

P1234

LOW HEPCIDIN/FERRITIN RATIO IN C282Y HOMOZYGOTES CORRELATES INVERSELY WITH TRANSFERRIN SATURATION AND NON-TRANSFERRIN BOUND IRON LEVELS

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Background and Aims: HFE Hereditary Haemochromatosis (HFE-HH) is characterised by deficient hepcidin production, a high serum ferritin and transferrin saturation, and subsequent formation of toxic non-transferrin bound iron (NTBI) with parenchymal iron overload. The purpose of this study was to determine the serum hepcidin:ferritin ratio across a cohort of different HFE genotypes presenting with raised ferritin levels, and to examine the relationship of this ratio with transferrin saturation, NTBI and LFTs.

Methods: Fasting blood samples were collected from 77 individuals referred with suspected iron overload to our centre. Transferrin saturation, ferritin and liver function tests were measured using standard laboratory methods. Serum hepcidin levels were measured using a combination of weak cation exchange chromatography and time of flight spectrometry, while NTBI levels were measured using a one step fluorescence-based assay. Data was analysed using SPSS version 18.

Results: The table depicts the study results. The hepcidin:ferritin ratio was significantly lower in C282Y homozygotes compared to compound heterozygotes and controls, despite similar ferritin levels across the groups. Transferrin saturation levels were significantly higher in C282Y homozygotes compared to compound heterozygotes and controls, and C282Y homozygotes had significantly higher NBTI levels compared to controls. ALT levels and age were not significantly different between groups. Within the C282Y homozygous cohort, the hepcidin:ferritin ratio strongly correlated with transferrin saturation ($r = -0.639$, $p = 0.001$) and NTBI ($r = -0.647$, $p = 0.001$), while ALT showed a moderate correlation with NTBI ($r = 0.431$, $p = 0.035$) and with the hepcidin:ferritin ratio (−0.405, $p = 0.049$).

	C282Y (1) n = 29	Compound heterozygote (2) n = 22	Controls (3)* n = 26	ANOVA/post hoc Tukey HSD (1) vs (2); (1) vs (3)
Hepcidin, nM	3.2 (2.2–3.7)	5.1 (3.1–7.2)	6.0 (4.6–10.6)	**<0.001**; **<0.001**
NTBI, μM	5.9 (4.7–7.1)	4.63 (4.1–5.69)	3.9 (3.1–4.8)	0.115; **<0.001**
Ferritin, μg/L	594 (364–1028)	405 (320–696)	470 (379–730)	0.273; 0.774
Trans Sat, %	72.4 (19.2)	47 (10)	38.9 (14.6)	**<0.001**; **<0.001**
ALT, IU/L	37 (25–51)	41 (28–56)	44 (20–59)	0.424; 0.285
Age, years	48.3 (10.3)	47.1 (12.1)	54 (11.2)	0.925; 0.150
Hepcidin:ferritin ratio	4.96 (3.7–6.3)	11.5 (7.1–18.5)	12.5 (9.4–21.2)	**<0.001**; **<0.001**

*6 H63D homozygotes, 6 C282Y heterozygotes, 7 H63D heterozygotes and 7 normal genotypes.

Conclusions: Low hepcidin:ferritin ratios are associated with high transferrin saturation and NTBI levels. The serum hepcidin/ferritin ratio may serve as a useful clinical tool to reflect the inadequate hepcidin response to increased iron loading. Moreover, increased NTBI levels may play a role in the suppression of hepcidin underlying the pathogenesis of HFE-HH.

P1235

HEREDITARY HEMOCHROMATOSIS: ROLE OF H63D HOMOZYGOSITY IN HEPATIC IRON OVERLOAD

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Background and Aims: Hereditary hemochromatosis (HH) is the leading genetic cause of iron overload. C282Y homozygotes account for 80–85% of patients with HH, however, H63D mutation may also play an important role. Current data argue both for and against the association between H63D homozygosity and hemosiderosis. Our aim is to analyse the effect of this polymorphism on iron metabolism and to determine if it predisposes carriers to develop a significant liver disease.

Methods: Retrospective analysis including 445 subjects studied from 1994 to 2012 who presented iron overload or family history of hemochromatosis. Genetic analyses were performed using allele-specific polymerase chain reaction amplification and data were obtained from medical records and laboratory database.

Results: We included 192 homozygous H63D, 109 homozygous C282Y, and 144 wild-type HFE subjects (without H63D nor C282Y mutations).

Mean ferritin and IST values for homozygous H63D were 372.1±385 mg/dl and 42.4±19.1%, respectively. Older patients had higher ferritin levels, indicating a trend towards gradual increase over time. Serum iron, AST, ALT, bilirubin, and GGT values were all within normal ranges in this group. Twenty-one H63D patients (10.94%) showed parenchymal iron overload (measured by biopsy or magnetic resonance), and seven (3.65%), signs of cirrhosis with portal hypertension. Only 11 cases (5.72%) showed iron overload in absence of other factors associated with hyperferritinemia.

C282Y homozygotes had significantly higher levels of ferritin (980.9 ng/mL) and IST (82.7%) than H63D group, and presented higher percentages of parenchymal iron overload (48.6%).

Subjects with wild-type alleles showed higher ferritin levels (595.6 ng/mL) than H63D homozygotes, but similar IST values, iron deposition, and portal hypertension prevalence.

Regarding possible confounding variables, wild-type subjects had the highest prevalence of non-inherited hyperferritinemia-related factors, confirming a secondary origin of iron overload in this group.

Conclusions: H63D homozygosity is related to increased levels of ferritin. Hepatic iron overload is present in 5.72% of these patients in the absence of other conditions associated to hyperferritinemia. However, H63D homozygosity doesn't determine

clinically significant iron overload in most cases. When these patients present analytical and/or histological evidence of iron overload, other hyperferritinemia-related factors should be investigated first.

P1236

IMPACT OF HIGH PORTAL PRESSURE ON RENAL HEMODYNAMICS IN CHILDREN WITH PORTAL HYPERTENSION

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Background and Aims: Hemorrhage from gastroesophageal varices in children with prehepatic portal hypertension is best controlled by various types of portosystemic shunt, but long-term follow up of children who have had splenorenal shunt surgery due to portal hypertension gave evidence for assuming the risk of renal venous hypertension (RVH).
The purpose of this study was to investigate the impact of portosystemic shunt surgery on renal blood
Methods: The results of 170 portosystemic shunt operations were followed from 2005 to 2014. 12 patients applied side to side splenorenal shunt, 12 children assessed the distal splenorenal shunt (DSRSh), 82 had central splenorenal shunt (CSRSh) with splenectomy. 44 iliacomesenterial anastomosis (IMA) and in 20 cases performed mesocaval anastomosis (MCA). Patients had a standard pre and postoperative work up including gastrointestinal endoscopy, Doppler ultrasonography (US), angiography, multislice computed tomography and MR imaging.
Results: On Doppler US at early and late postoperative periods PI and RI of left renal artery remained at high numbers (PI = 1.48±0.17 and RI = 0.72±0.19, $p \leq 0.05$ respectively) after the CSRSh with splenectomy. After DSRSh, these signs haven't been detected. 11 (13.4%) patients after CSRSh on Doppler US revealed signs of impeded venous outflow on the left renal vein (LRV). 8 patients after IMA on US Doppler and CT and MRI angiography revealed dilated left testicular and ovarian veins (Figure 1), with retrograde blood flow in them, which clinically manifested as left flank pain, macro- and microhematuria, varicocele and ovaricocele
Conclusions: Portosystemic shunt surgery is highly effective treatment for children with prehepatic portal hypertension. But study shows that total shunts such as central splenorenal shunt with splenectomy and iliacomesenterial anastomosis more negatively effect on hemodynamics of left kidney. Shunting the large amounts of blood from a system of high pressure to a low manifests as clinical signs of renal venous hypertension.

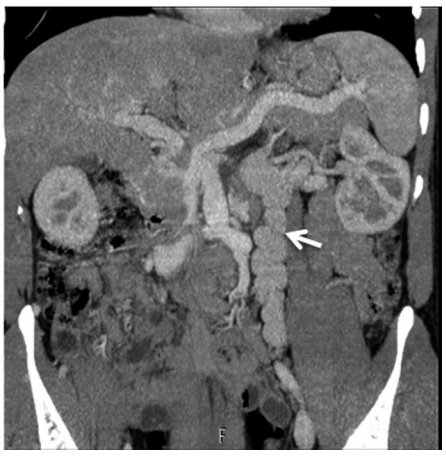

Figure 1. Fourteen-year-old boy with extrahepatic portal hypertension, after IMA. MSCT angiogram reveals enlarged and tortuous spermatic vein (arrow) draining to inferior cava through left renal vein.

P1237

THE MODIFIED IRON AVIDITY INDEX: A PROMISING PHENOTYPICAL PREDICTOR IN HFE-HAEMOCHROMATOSIS

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Background and Aims: Phenotypes of the HFE-related haemochromatosis vary considerably, which makes it difficult to identify which patients need maintenance phlebotomy therapy, and if so, how often. Besides phlebotomy therapy has side effects; therefore, it would be of great value to have a tool to predict the phenotype, to be able to individualize therapy. The aim of this retrospective study is to determine if the iron avidity index (IAI) is a good phenotypical predictor of the number of phlebotomies during maintenance treatment (NPDMT) needed per year in patients with C282Y homozygous haemochromatosis.
Methods: Patients with hereditary haemochromatosis homozygous for C282Y on maintenance treatment, in the Atrium Medical Centre, Heerlen, the Netherlands, for at least one year, were included. The IAI was calculated by dividing ferritin level at diagnosis by age at diagnosis. Due to skewed distribution, IAI was natural logarithmic (LN) transformed.
Linear regression analysis was used to assess the association between the IAI and NPDMT and to assess influence of current age, age at diagnosis, sex and PPI use on this association.
Furthermore, Pearson correlation was used to analyse the correlation between the modified IAI and NPDMT per year. The results were analysed using IBM SPSS Statistics 22 software (IBM Inc., Armonk, NY, USA).
Results: In total, 95 patients (60% men) with a mean age of 62 (±10.7) years, were included in the analysis. The IAI is showing a significant positive relation with NPDMT per year ($r = 0.299$, $p = 0.002$). Linear regression analysis showed the confounding effect of sex on the relationship between IAI and NPDMT per year. PPI use was independently associated with NPDMT. A modified Iron Avidity Index, adjusted for sex, was calculated and showed a significant positive correlation with NPDMT ($r = 0.343$, $p = 0.000$) (see Figure 1).

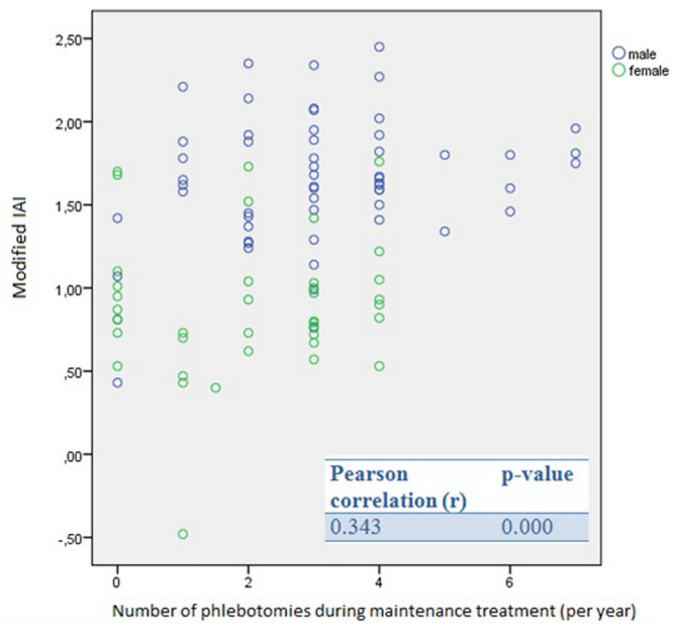

Figure 1. Scatterplot of the modified IAI vs. number of phlebotomies during maintenance treatment, with a Pearson correlation of 0.343; showing that women need less phlebotomies per year during maintenance treatment.

Conclusions: The modified IAI, adjusted for sex, is a promising index for predicting the yearly number of phlebotomies during maintenance treatment as an expression of the phenotype of patients with hereditary haemochromatosis homozygous for C282Y. Due to the retrospective design of the study, information on factors, such as weight, BMI and diet, were lacking and are probably of importance because of the fact that HFE related haemochromatosis is a heterogeneous disorder. Future studies should be performed to refine this index to make it into a useful tool to predict the number of phlebotomies in maintenance therapy more accurately.

P1238

INTERMEDIATE FAMILIAL INTRAHEPATIC CHOLESTASIS (IFIC): PHENOTYPIC SPECTRUM WITHIN THE BRIC–PFIC SPECTRUM

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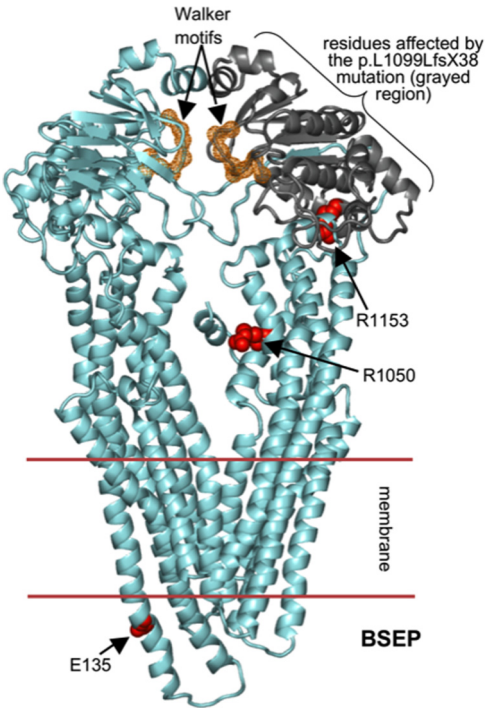
Background and Aims: Benign recurrent intrahepatic cholestasis (BRIC) and progressive familial intrahepatic cholestasis (PFIC) belong to a *spectrum* of autosomal liver disorders: familial intrahepatic cholestases (FIC). Their differentiation is based on genetic and phenotypic presentation. Clinical transition between BRIC and PFIC is rarely reported. We hypothesize that patients with a compound heterozygosity for BRIC and PFIC mutations belong to an intermediate form of familial intrahepatic cholestasis (IFIC).
Methods: Two cases, a male (case 1), 11 year-old, with intermittent jaundice since 1 month-old and a female (case 2), 5 year-old, with intractable pruritus and jaundice, since 5 months old, underwent liver biopsies, which were analyzed by histology and immunohistochemistry (IHC) with an anti-BSEP antibody. *ABCB11* gene was sequenced. To define the mutation pathogenicity, the homology modeling was done (Figure).



Results: In case 1, liver biopsy showed preserved architecture and mild signs of intrahepatic cholestasis. Genetic analysis showed p.E135K (BRIC mutation) and p.L1099LfsX38 (new mutation). The homology modeling revealed that p.E135K has an effect on the N-glycosylation interfering with stability, function and intracellular trafficking. Conversely, the p.L1099LfsX38 causes the loss of most of the residues forming the 2nd ABC transporter domain, abolishing the protein function (PFIC mutation). In case 2, three liver biopsies displayed progression of cholestasis and IHC revealed persistent preserved BSEP canalicular staining. Genotyping showed p.R1050C (BRIC mutation) and p.R1153H (PFIC mutation). The p.R1153H should impair the protein function influencing the structure of the Walker motif in the 2nd ABC transporter domain of BSEP. The p.R1050C, interacting with a site of ubiquitination, will increased BSEP degradation.
Conclusions: Familial intrahepatic cholestasis is distinguished into BRIC and PFIC. A clinical progression from benign to severe form is rarely described. Our cases suggest the existence of a *de novo* form of Intermediate FIC, with an intermediate phenotype between the benign phenotype (BRIC) and the more severe (PFIC), genetically supported by a compound heterozygosity (BRIC-PFIC). Absence of phenotype-genotype correlation, at least in our cases, would suggest the possibility of a IFIC *spectrum,* depending on mutation severity.

P1239

ASSESSMENT OF DIET AND PHYSICAL ACTIVITY IN PAEDIATRIC NON-ALCOHOLIC FATTY LIVER DISEASE PATIENTS: A UK CASE CONTROL STUDY

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is now the most common cause of chronic liver disease in children with an estimated prevalence of 10% in the general paediatric population increasing to 80% in obese children. The aim of this study was to characterise the habitual diet and activity behaviors of children with biopsy-confirmed NAFLD compared to obese children without liver disease.
Methods: Biopsy proven paediatric NAFLD cases and ultrasound clear obese controls (OC), recruited from King's College Hospital specialist paediatric liver and obesity clinics respectively completed 24-hour dietary recall, a Physical Activity Questionnaire (PAQ), a Dutch Eating Behavior Questionnaire (DEBQ) and a 7-day food and activity diary (FAD) in conjunction with wearing a pedometer. Personal diet and lifestyle feedback was provided with the goal of weight reduction at 6-month follow-up.
Results: 21 NAFLD and 8 OC have completed the study to date. Groups were well matched for age, gender and baseline anthropometrics with the majority of patients having a BMI over the 99.6th\ centile. The NAFLD group were predominantly Caucasian (80%) while OC group were largely Black (78%) ($p < 0.01$). 24-hour recall data highlighted NAFLD patients had higher intakes of saturated fatty acids (SFA) and iron when comparing dietary recommended values ($p = 0.048$ and $p = 0.021$ respectively), while the 7-day FAD demonstrated higher dietary intakes of vitamin D in the OC compared to NAFLD (3.7 μg/d versus 2.1 μg/d; $p = 0.05$). No significant differences in absolute intakes of fructose, or Vitamins C or E were identified. Under-reporting was prevalent within the sample (NAFLD: 65%, OC: 75%; $p = 0.61$). In addition, NAFLD patients showed a greater number of steps taken (NAFLD: 8,414, OC: 5,965; $p = 0.02$) despite both groups not meeting the recommended 10,000 steps a day.

Conclusions: This is the first study to assess dietary and lifestyle activity in a UK paediatric NAFLD population. While clear differences were not identified between groups, issues of sample size, under reporting and confounding by ethnicity have impacted on the findings and are being addressed in the ongoing study. Development of NAFLD-specific diet and lifestyle guidelines are currently not supported. The current study demonstrates that the paediatric population is failing to conform to standard recommendations for both diet and physical activity, and should therefore remain the focus of clinical management.

P1240
CLINICAL, BIOCHEMICAL AND HISTOPATHOLOGIC PROFILE OF SUBJECTS WITH GLYCOGENIC HEPATOPATHY (MAURIAC SYNDROME)

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Background and Aims: Glycogenic hepatopathy is a syndrome of elevated transaminases and liver enlargement in type-1 diabetes patients. Since its description, there have been mostly case reports discussing this condition. We aimed to conduct a case series to describe clinical, biochemical and histo-pathologic profile of these patients.

	Overall (n = 36)	Adults (n = 24)	Pediatric (n = 12)
Male	8 (22.2%)	5 (20.8%)	3 (25%)
Age at presentation (years) [a]	20.9±5.8	23.3±5.7	16.3±1.76
Height (cm) [b]	166.8 (161.8, 169.5)	168 (162.4, 171.6)	161.5 (148.8, 164.9)
Weight (kg) [a]	64.4±13.3	66±9.7	61.1±18.8
Body mass index (BMI; kg/m^2) [a]	23.5±3.5	23.4±2.7	23.8±4.8
Percentile height for age [b]	30 (8.5, 49)	n/a	30 (8.5, 49)
Percentile weight for age [b]	78.5 (43.5, 83.8)	n/a	77 (62.5, 80.8)
BMI for age [b]	76 (53, 84)	n/a	81.5 (72.3, 89.8)
Clinical features			
Abdominal pain	23 (63.9%)	14 (58.3%)	9 (75%)
Vomiting	6 (16.7%)	6 (25%)	0
Abdominal distention	6 (16.7%)	2 (8.3%)	4 (33.3%)
Splenomegaly	2 (5.6%)	0	2 (16.7%)
Hepatomegaly	22 (61.1%)	12 (50%)	10 (83.3%)
Type-1 DM	36 (100%)	24 (100%)	12 (100%)
Age at diagnosis of DM (years) [b]	8.5 (5, 12)	9.5 (5.5, 12.8)	7.5 (5, 9.25)
Duration of DM (years) [a]	11.6±5.6	13.3±5.8	8.7±3.6
Requires insulin	35 (97.2%)	23 (95.9%)	12 (100%)
Episodes of DKA	22 (61.1%)	13 (54.2%)	9 (75%)
Imaging findings			
Splenomegaly	0	0	0
Hepatomegaly	28 (77.8%)	19 (79.2%)	9 (75%)
Laboratory findings			
HBa1c (%) [a]	11.2±2.4	11.5±2.6	10.7±2
Aspartate transaminase (U/L) [b]	289 (139, 594)	313 (246, 770)	157 (114, 254)
Alanine transaminase (U/L) [b]	245 (126, 386)	315 (189, 386)	145 (77, 300)
Alkaline phosphatase (U/L) [b]	196 (145, 288)	193 (149, 273)	243 (142, 287)
Gamma glutamyl transferase (U/L) [b]	97 (66, 365)	181 (123, 366)	77 (66, 271)
Total bilirubin (mg/dL) [a]	0.5±0.3	0.5±0.4	0.5±0.2
Direct bilirubin (mg/dL) [a]	0.2±0.3	02±0.3	0.2±0.1
Serum albumin [a]	3.9±0.6	3.7±0.5	4.3±0.4
INR [a]	0.86±0.07	0.88±0.07	0.82±0.04
Total cholesterol (mg/dL) [a]	266±95	281±112	242±56
LDL cholesterol (mg/dL) [a]	112±38	116±39	107±38
HDL cholesterol (mg/dL) [a]	51±24	52±24	50±24
Triglycerides (mg/dL) [b]	291 (188, 507)	318 (188, 572)	259 (202, 361)
Biopsy			
Performed	20 (55.5%)	16 (66.6%)	4 (33.3%)
Inflammation	3 (15%)	3 (18.75%)	0
Megamitochondria	3 (15%)	2 (12.5%)	1 (8.33%)
Fibrosis	2 (10%)	1 (6.25%)	1 (8.33%)
Follow up			
Resolved transaminases	18 (50%)	9 (37.5%)	9 (75%)
Resolving transaminases	3 (8.3%)	2 (8.3%)	1 (8.3%)
No follow up	15 (41.6%)	13 (54.2%)	2 (16.7%)

[a] Mean ± standard deviation. [b] Median (interquartile range).

Methods: Electronic medical records at Mayo Clinic, Rochester were searched from January 1, 1998 to January 1, 2014 using the term "Mauriac syndrome" and "Glycogenic hepatopathy" and reviewed in detail. Demographic, clinical, biochemical and histopathologic data was abstracted.
Results: Results are described in the table. 36 patients were diagnosed with glycogenic hepatopathy – 24 adults and 12 pediatric patients. 20 had liver biopsy and 16 were clinically diagnosed. 28 (77.8%) cases were female. Only 2 out of 12 (16.7%) pediatric patients, both from the clinically diagnosed group, had percentile height, weight and BMI for age below the third percentile. Abdominal pain was the most common symptom [23 (63.9%)] and hepatomegaly was seen in 28 (77.8%) cases on imaging. All patients had poor control of diabetes (HBA1c – 11.2±2.4). Transaminases were elevated from 3 to 20 times the upper limit of normal.
Conclusions:
- Growth retardation is uncommon in glycogenic hepatopathy.
- Majority of cases present with abdominal pain, hepatomegaly, transaminase elevation, hypertriglyceridemia, without any synthetic liver dysfunction.
- Liver biopsy rarely shows fibrosis, inflammation or megamitochondria.
- On follow up, majority of individuals with follow up data had resolution of transaminases with improved glycemic control.

P1241
JAK/STAT PATHWAY AND SUSCEPTIBILITY TO HEPATITIS B VIRUS INFECTION AND RELATED LIVER DISEASES

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Background and Aims: Hepatitis B virus (HBV) infects approximately 350 million people worldwide and is the leading cause for chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. The activation of JAK/STAT pathway by cytokines and growth factors is related to different processes such as cell proliferation, differentiation and apoptosis. Several protein members of JAK/STAT pathway including SOCS1, SOCS3, STAT1, STAT3, and STAT5 have been demonstrated to associate with the HBV infection and modulate the outcomes of liver diseases. We investigated the influence of polymorphisms in the JAK/STAT pathway gene members including Cytokine-inducible SRC homology 2 (SH2) domain protein (*CISH*), the signal transducers and activators of transcription 4 (*STAT4*) and the suppressors of cytokine signalling 3 (*SOCS3*) on HBV infection and HBV-related liver diseases.
Methods: We utilized a Vietnamese cohort (n = 1084) clinically classified into different subgroups including chronic hepatitis B carriers, liver cirrhosis, hepatocellular carcinoma and healthy controls to investigate the effect of the reported variants. The genotyping of investigated variants was performed by FRET real-time PCR and sequencing. A multivariate regression analysis was performed to investigate potential effect of studied variants and to exclude the confounding effects of other factors such as age and gender. We also analyzed the influence of studied variants on the disease outcomes represented by different clinical parameters.
Results: The first results showed that *CISH* variants −292A/T were significantly associated to HBV infection (Allelic: OR: 1.22, 95% CI 1–1.49; $P = 0.04$; Recessive: OR: 1.69, 95% CI 1.23–2.54; $P = 0.007$). A gene dose effect for the risk allele *−292T* was also observed ($P = 0.04$). In addition, the *STAT4* variant (rs7574865) was marginally

associated with HCC susceptibility in CHB carriers in allelic and recessive genetic models (OR = 0.84, 95% CI 0.7–0.99, P = 0.048 and OR = 0.7, 95% CI 0.5–0.99, P = 0.047).
Conclusions: Our findings significantly contribute to understand the functional roles of JAK/STAT pathway member proteins during HBV infection, pathogenesis and progression of liver diseases.

P1242
ETHNIC DIFFERENCES IN PAEDIATRIC NON-ALCOHOLIC FATTY LIVER DISEASE

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is now the most common cause of liver disease in children, with an estimated prevalence of 5–10%. The majority of published data has involved Caucasians. Data is lacking regarding the phenotypic differences of NAFLD in children of Asian origin.
Methods: We performed a retrospective review of children consecutively diagnosed with NAFLD at a tertiary referral hepatology service in the UK, between 2003 and 2014. Patients had been referred from either primary care physicians or general paediatricians with incidental asymptomatic abnormal liver function tests (LFTs) and/or fatty liver on ultrasound scan (USS). Patients with inadequate data or with secondary fatty liver disease were excluded. Patients were divided by ethnicity into Asian and Caucasian. All values are (±SD), unless stated.
Results: 100 patients with primary NAFLD were identified. 59/100 were Caucasian and 41/100 were of Asian origin, of whom, 73% (30/41) were Pakistani. There was male predominance in both groups, 73% (43/59) in Caucasian and 78% (32/41) in Asians. Mean ages at presentation were 12.6±2.5 and 11.7±2.8 years in Caucasian and Asian groups, respectively.
Asian patients had a significantly lower body mass index (BMI) centile than Caucasian (91.2±9.5 vs. 96.1±5.1, $p < 0.05$). There were no differences in LFTs between Asian and Caucasian patients, ALT (87.8±60.9 vs. 94.3±84.5 IU/L) and bilirubin (9.4±7.6 vs. 9±6.5 μmol/L). 61% (25/41) Asian patients had an elevated gamma glutamyl transferase, compared to 60% (35/59) Caucasian patients. Markers of the metabolic syndrome were similar in both groups: total cholesterol 4.4±1 vs. 4.4±1 mg/L, triglycerides 2.0±1.4 vs. 2.2±1.4 mg/L, and HbA1c 38.6 vs. 39.1±7.4 mmol/mol.
20 patients had undergone biopsy. 33% (2/6) demonstrated moderate-severe fibrosis in Asian patients, compared to 21% (3/14) Caucasian patients ($p = 0.61$).
Conclusions: Asian children with NAFLD have similar disease severity as their Caucasian counterparts, despite a significantly lower BMI centile at presentation. Referring clinicians and risk stratification scores require a greater awareness of ethnic differences in adiposity in paediatric NAFLD.

EU Public health

P1243
BIRTH COHORT DISTRIBUTION AND SCREENING OF VIREMIC HCV INFECTIONS IN LUXEMBOURG

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Background and Aims: In Luxembourg, the majority of viremic hepatitis C virus (HCV) infected individuals are thought to have already been diagnosed (85%). Additionally, nearly 100 viremic cases are newly diagnosed annually. As the undiagnosed population dwindles, identifying new cases becomes increasingly difficult (Wedemeyer 2014). This analysis seeks to understand if targeted birth cohort screening strategies can improve detection rates.
Methods: A previously described HCV disease burden model was populated with Luxembourgish assumptions regarding HCV prevalence, viremic rate and age distribution (Razavi 2014). The viremic population was aged to 2013 accounting for mortality and cure rates, and was stratified according to birth cohort. The number of screenings required to identify one viremic case was calculated as follows:
1/[(viremic HCV prevalence)·(1 − diagnosis rate)].
Costs (expressed in €) associated with diagnosing (anti-HCV serology and HCV RNA qualitative) and informing treatment (genotype and viral load) for one viremic case were estimated as follows:
€8·(anti HCV tests administered) + €65·(HCV RNA tests administered for anti HCV positive cases) + €228·(treatment informing tests administered for HCV RNA positive cases).
Results: More than 50% of the viremic HCV infected population was estimated to be born between 1965 and 1984, with 75% born between 1960 and 1989. Random screening of the general population would require 1,120 anti-HCV tests (in addition to further confirmatory tests) to diagnose one new HCV case, while targeted screening in the 1960–1989 cohort (25–50 years of age) could diagnose one new case per 671 persons screened. Targeted screening in the 1960–1989 cohort would cost about €8,170 per positive diagnosis, as compared with a €13,460 cost for random screening. Screening within 5-year age cohorts could find anywhere from one case per 569 persons screened (1975–1979 cohort, 15% of the viremic population) to one case per 236,680 persons screened (2010–2014 cohort, <1% of the viremic population).
Conclusions: Considering only the direct cost of diagnosis, the most efficient birth-year screening strategy would particularly target persons born between 1960 and 1984 (25–50 years of age), as this cohort accounts for 75% of viremic cases and requires screening of only 671 individuals to identify one viremic HCV infected case. Targeted birth cohort screening is more efficient and cost effective than random screening.

P1244

PREVALENCE AND CLINICAL OUTCOME OF POST TRANSFUSION HEPATITIS C IN YOUNG AND ADULTS

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Background and Aims: The prevalence of chronic hepatitis C in Sweden is estimated to 0.5%. Before 1991 blood transfusion was a common route of transmission. The primary aim of this study was to estimate anti HCV prevalence in individuals receiving blood transfusions in an urban area during that period. The secondary aim was to study the effect of age at transfusion on liver disease and treatment outcome.
Methods: This is a single centre retrospective analysis of anti HCV tested individuals during a national screening campaign in 2008–2010 of recipients of blood transfusions 1965–91 in an urban area. Subjects were informed through media and encouraged to perform anti HCV testing by their general physician. All anti HCV positive subjects were referred to Karolinska University Hospital for further investigation. Inclusion criterion for this study was blood transfusion as the most likely mode of transmission. Subjects with ongoing or a history of drug abuse were excluded. Data on age at transfusion, age at diagnosis, HCV genotype, IL28B genotype, viral load, fibrosis score, liver histology and antiviral treatment was recorded.
Results: 134 of 7473 (1.8%) tested individuals were anti HCV positive and of those 102 were HCV RNA positive resulting in a prevalence of chronic hepatitis C infection of 1.4%. In 99 patients data was retrieved showing that 71% were female, 45% of the patients were older than 61 y at diagnosis. Obstetric or gynecological intervention were the most common causes of transfusion. The rate of advanced liver damage was 18% (n = 56). Patients younger than 19 y of age at transfusion (group1, 23% of the cohort) were significantly more often started on antiviral treatment compared to adult patients (group 2), 65% vs 29% $p < 0.001$. No significant correlation was found between treatment outcome and gender, age at transfusion or IL28B gt.
Conclusions: This study found an anti HCV prevalence of 1.8% which is considerably higher than previous estimations. Most patients acquiring HCV through transfusions are female, but mostly elderly patients were identified in a national campaign. In this study, patients infected during childhood were more likely to receive antiviral treatment than individuals with post transfusion hepatitis C contracted later in life. Additional data on the hepatitis C epidemic in Sweden are needed regarding prevalence and age distribution. If treatment coverage is higher in younger individuals this population should be targeted for general screening.

P1245

PUBLIC HEALTH IMPACT OF HCV SCREENING AND TREATMENT IN THE FRENCH BABY-BOOMER POPULATION

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Background and Aims: Because of the high prevalence of chronic hepatitis C in baby-boomers (born between 1945 and 1965) in many developed countries, screening and treatment of this population is of public health importance. This study examined the projected impact of screening a French baby-boomer (FBB) population for hepatitis C virus (HCV), followed by one of four treatment strategies for naïve genotype (GT) 1 patients: (1) no treatment; (2) interferon (IFN)-based direct-acting antiviral (DAA) regimens in fibrosis stages F2–F4 (current standard of care); (3) IFN-free all-oral DAA regimens in stages F2–F4 or (4) IFN-free all-oral DAA regimens in stages F0–F4.
Methods: A sequential, multi-cohort, health-state-transition model projected the number of FBBs with HCV achieving a sustained virologic response 12 weeks after treatment (SVR_{12}) or progressing to decompensated cirrhosis (DCC), hepatocellular carcinoma (HCC), liver transplant (LT) and liver-related death (LrD) from 2015 to 2035. Epidemiology and mortality data were extracted from the median population projection by the French National Demographic Institute. The proportion of FBBs screened for HCV was projected to increase from 57% in 2015 to 72%/78%/85% (low/medium/high screening scenarios) in 2035, with a new cohort of patients being diagnosed each year. SVR_{12} rates were extracted from clinical trials. A HCV prevalence estimate of 0.5% (61% GT 1) and transition probabilities across health states were obtained from published literature.

Table: Projected public health impact (medium HCV screening scenario 2015–2035)

	Time period				
	2015–2019	2020–2024	2025–2029	2030–2035	Overall
Number of newly diagnosed patients from screening					
F0–F1	727	525	331	160	1,743
F2–F3	306	319	271	169	1,066
CC	66	59	51	35	212
Total	1,099	904	653	364	3,020
Total, low scenario	770	655	501	324	2,250
Total, high scenario	1,428	1,153	804	405	3,790
Number of life years					
No treatment					
SVR_{12}	0	0	0	0	0
DCC	9	42	84	153	288
HCC	7	36	76	143	262
LT	0	3	10	24	38
LrD [a]	3	17	35	65	120
IFN-based DAAs for F2–F4					
SVR_{12}	725	2,891	5,131	8,158	16,906
DCC	1	2	2	2	7
HCC	1	2	2	2	6
LT	0	0	0	1	1
LrD [a]	0	1	1	1	3
IFN-free DAAs for F2–F4					
SVR_{12}	743	2,921	5,164	8,193	17,020
DCC	0	0	0	0	2
HCC	0	0	0	0	2
LT	0	0	0	0	0
LrD [a]	0	0	0	0	1
IFN-free DAAs for F0–F4					
SVR_{12}	2,194	7,053	10,447	14,241	33,935
DCC	0	0	0	0	2
HCC	0	0	0	0	2
LT	0	0	0	0	0
LrD [a]	0	0	0	0	1

DCC, Decompensated cirrhosis; F, Fibrosis stage; HCC, Hepatocellular carcinoma; LT, Liver transplantation; LrD, Number of liver-related deaths; SVR_{12}, Sustained virologic response 12 weeks after treatment.
[a] Measured as number of cases.

Results: The low, medium and high HCV screening scenarios demonstrated that 2,250, 3,020 and 3,790 new FBBs would be diagnosed with HCV from 2015 to 2035 (see Table). In the medium screening scenario, the number of life years lived with SVR_{12} (and patients achieving SVR_{12}) during 2015–2035 would be 16,906 (1,740) with the current standard of care, 17,020 (1,747) with all-oral, IFN-free DAAs for F2–F4, and 33,935 (2,952) with all-oral, IFN-free DAAs for F0–F4. The number of patient-years lived in DCC, HCC or LT during 2015–2035 would be 587 (120 LrDs) without treatment, 14 (3 LrDs) with the current standard of care, and 4 (1 LrD) with IFN-free, all-oral DAAs for F2–F4 or F0–F4.
Conclusions: An enhanced screening policy coupled with broader access to IFN-free, all-oral DAAs will diminish the future burden of HCV in the FBB population. Our analysis suggests that IFN-free, all-oral DAAs for F0–F4 could double the number of years FBBs live with SVR_{12} compared to the current standard of care.

P1246

ABDOMINAL ULTRASONOGRAPHY GUIDANCE REDUCES THE RISK OF COMPLICATIONS AND IMPROVES SUCCESS RATE AFTER LIVER BIOPSY: RESULTS OF A META-ANALYSIS COMPARING THE USE OF ULTRASOUND GUIDANCE TO THE "BLIND METHOD"

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Background and Aims: Percutaneous liver biopsy (LB) remains essential for histological diagnosis of many diffuse liver diseases. Two techniques can be used: "blind" liver biopsy (B-LB) and ultrasound-guided liver biopsy (US-LB). Reports on usefulness of US guidance are conflicting. The present meta-analysis aims to assess whether ultrasonography guidance effectively reduces the occurrence of complications and improve the quality of the material harvested.
Methods: Studies comparing the two techniques were selected by 3 independent searchers on PubMed. 24 pooled analyzes were conducted regarding the following endpoints: pain, complications (including anxiety, hypotension, bleeding and injury of the biliary tract), mortality, sample size, number of portal tracts and failure rate as assessed by histologic examination. Statistical analyses were performed according to the random effect model (Der Simonian and Laird) with determination of weight-adjusted odds ratios (ORs).
Results: 17 publications published as full paper in peer review journals including three randomized controlled studies were included. Data from 24,630 biopsies (14,603 B-LB and 10,027 US-LB) in 24,180 patients were analysed. Compared with B-LB, US-LB was significantly associated with lower failure rates (OR = 0.32; 95% CI: 0.16–0.62; $p < 0.0001$), less pain (OR = 0.55; 95% CI: 0.44–0.70; $p < 0.0001$) and fewer complications (all events combined) (OR = 0.54; 95% CI: 0.37–0.79; $p < 0.0001$). When restricting the analyses to randomized controlled trials, the benefit of ultrasound guidance was maintained for pain (OR = 0.51; 95% CI: 0.34–0.77; $p < 0.0001$) and for overall complications (OR = 0.37; 95% CI: 0.20–0.72; $p = 0.003$). The low number of events did not allow powerful analyses for each complication or mortality. The analyses regarding sample size and number of portal tracts provided heterogeneous results.
Conclusions: This meta-analysis indicates the superiority of LB with ultrasound guidance compared with "blind" LB. The risk of pain and complications are reduced by 50% and the success rate is improved by 68%. These results support the recommendation of a systematic ultrasound-guidance for all percutaneous LB.

P1247

UNEXPECTED HIGH PREVALENCE OF HCV-VIRAEMIA (HCV RNA+) IN THE EMERGENCY DEPARTMENT (ED) OF A LONDON HOSPITAL – SHOULD WE BE SCREENING FOR HCV IN ED ATTENDEES?

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Background and Aims: The UK national public health authority, Public Health England, recently recommended a rapid scale-up in diagnosis and treatment of hepatitis C virus (HCV) infection in order to reduce the substantial HCV-related healthcare burden. It is estimated that up to 50% of HCV infected individuals in the UK remain undiagnosed. While excellent treatments now exist, making the diagnosis is the key to curing HCV, both for individuals and for public health. 1 in 4 people in the UK attend an Emergency department (ED) each year. However, HCV testing is not routine and data on HCV prevalence in ED settings are sparse. Local prevalence data are crucial in informing public health strategy for HCV.
Aim: To estimate the HCV RNA+ prevalence and cost per diagnosis per viraemic infection in ED attendees.
Methods: We conducted an unlinked anonymous HCV seroprevalence study using residual biochemistry samples from 997 Royal London hospital ED attendees over 18 years during a 12 day period between 4/9/14 and 16/9/14. Samples were anonymised except for age, gender and ethnicity and tested for HCV antibody (Ab) using an automated EIA (Architect, Abbott). Reactive samples were further tested for HCV RNA (COBAS Amplicor, Roche). Anonymisation of samples and HCV testing were double blinded.
Results: 997 samples were tested for HCV Ab, overall HCV seroprevalence was 2.6% (26/997) with 1.2% (12/997) HCV RNA+. Median age of RNA+ was 47 y (age unknown in 80). 66.7% (8/12) of RNA+ were male. 75% (9/12) RNA+ individuals were aged 25–54 y (RNA+ 9/441; 2.0%). There was a substantial variation in age–sex specific RNA+ prevalence peaking at 48% (3/63) in males 35–44 y, compared to 0% (0/136) <35y ($p = 0.0614$) and 1.4% (4/278) ≥45 y ($p = 0.2415$). Despite ethnic diversity within ED attendees with 30% being Asian (300/997) and 31% white British (311/997), 58% (7/12) of RNA+ were in white British attendees (2.3% RNA+ in white British vs 0.3% in Asians; $p = 0.0766$). Assuming cost for HCV Ab is £6 and HCV-RNA is £40/test, screening ED attendees aged 25–54 could identify 75% of RNA+ at a cost of £360 per viraemic infection. This would likely yield the most favourable benefit/cost ratio.
Conclusions: We found unexpectedly high levels of active HCV infection in screening ED attendees in this ethnically diverse large urban London hospital. The distinct local epidemiological profile of RNA+ attendees suggests that routine screening of ED attendees aged 25–54 years could be an effective strategy.

P1248

SUCCESSFUL MANAGEMENT OF PRECARIOUS POPULATION AFTER SYSTEMATIC HBV TESTING IN FRANCE: A PROSPECTIVE COHORT STUDY

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Background and Aims: In France, HBV infection is two to six times more prevalent among precarious individuals, especially migrants. However, their conditions of life make them unlikely to have access to screening and care. *Aims:* To offer precarious individuals HBV testing and access to care when positive.
Methods: Between May 2007 and May 2014, 149 (6.7%) out of 2,223 consecutive precarious adults attending two primary healthcare settings in Créteil, France, were tested HBsAg+. 95% were unaware of their status. 66% were male, mean age was 32 years (±9). Two were French, and 152 were migrants (sub-Saharan African 139, Asian 5, Eastern European 5, Latin American 2, Mediterranean area 1). Sixty-eight were asylum seekers and 72 illegal immigrants. All were offered referral to specialist.
Results: 140 HBsAg+ patients (91%) kept their appointment with a specialist, within a median of 33 days after testing, and 134 of them (96%) underwent biological and virological evaluations: 7 were HBeAg+, 57 had HBV DNA level >2,000 IU, 21 had >20,000 IU, and 26 had ALT > upper limit of normal. Co-infections were observed in 9 cases (HIV 5, HCV 2, HDV 2). Chronic active hepatitis was diagnosed in 27 patients, among which 1 cirrhosis and 2 patients were immune-tolerant. When needed, 14 out of 19

patients had access to antiviral therapy. The median follow-up was 14 months and currently, 83 patients (59%) are still followed-up.
Conclusions: Testing and referral were well accepted among this precarious population, in the context of a coordinated program that enables them to have access to optimized care.

P1249
ALCOHOLIC LIVER DISEASE AND LIVER CANCER AS PUBLIC HEALTH ISSUES IN THE BURDEN OF LIVER-RELATED MORTALITY IN THE LAST 15 YEARS IN BRAZIL

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Background and Aims: Liver-related mortality (LRM) has been increasing in the last decades worldwide. The aims of this study were to evaluate the global and specific LRM rates in the last 15 years in Brazil.
Methods: We analyzed mortality data in the Brazilian Unified Health System Information Technology Department (DATASUS) database from 1998 to 2012. Death causes were classified according to the 10th revision of the International Classification of Diseases (ICD-10). The ICD-10 codes for LRM were: viral hepatitis [B15-B19]; liver cancer [C22]; alcoholic liver disease [K70]; liver fibrosis and cirrhosis [I85, K73, K74] and others liver diseases [K72, K75, K76]. Population habitants' statistics were also available by the Brazilian Institute of Geography and Statistics (IBGE). Mortality rates were calculated by the ratio between absolute number of deaths and current estimated population and were expressed as death rates per 1,000 or 100,000 inhabitants.
Results: Overall mortality rates ranged from 5.76 to 6.09 deaths per 1,000 persons from 1998 to 2012. Cardiovascular disease, non-liver cancer and external causes were the top ranking death causes. A total of 482,238 people died due to liver diseases in the last 15 years, representing the 7th known cause of death. LRM rates [per 100,000 habitants] has been increasing from 15.7 to 19.7 from 1998 to 2012 and it was higher in males compared to females (26.1 vs 9.7) and higher in the South-East region compared to the North region (20.3 vs 11.3) in the last 15 years. Liver fibrosis and cirrhosis (31%), alcoholic liver disease (23%) and liver cancer (22%) were the most frequent liver-related death causes in this period. In individuals older than 40 years, 23,304 people died from liver cancer (10.5 per 100,000) from 1998 to 2002 compared to 29,969 (11.7 per 100,000) from 2003 to 2007 and 37,759 (12.0 per 100,000) from 2008 to 2012. Following the same trend, in people older than 40 years, deaths [per 100,000 habitants] due to alcoholic liver disease has been increasing: 10.6 (23,641 deaths) from 1998 to 2002; 12.5 (32,037 deaths) from 2003 to 2007 and 13.3 (41,721 deaths) from 2008 to 2012. On the other hand, death rates [per 100,000 habitants] from liver fibrosis and cirrhosis have been decreasing in Brazil in the last 15 years [18.3 (1998–2002); 16.9 (2003–2007); 14.5 (2008–2012)].
Conclusions: Liver-related death rates, especially due to liver cancer and alcoholic liver disease, have been increasing in the last 15 years in Brazil.

P1250
LOW INCIDENCE OF REINFECTION WITH HEPATITIS C VIRUS AFTER SUCCESSFUL TREATMENT IN MONTREAL

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Background and Aims: In Montreal the incidence rate of HCV infection is estimated at 26/100 py among the IDU population. Though treatment uptake among HCV infected patients remains low, the latter is likely to change given the recent development of promising INF-free treatments. HCV reinfection has been reported both in UDI and MSM patients but the extent to which reinfection occurs is unknown. The aim of this study was to evaluate the incidence of reinfection in a clinical cohort of HCV treated patients.
Methods: HEPVIRAC's (Hepatite Viral – l'Actuel's Cohort) patients with sustained virological response (SVR) to HCV treatment were included in this study. Censoring date was either the date of HCV reinfection or the date of the last negative HCV RNA test. Reinfection was defined as detectable HCV RNA with or without ALT elevation. Date of reinfection was estimated as the midpoint between the last negative and first positive HCV RNA tests. The rate of reinfection was calculated using the number of person-years of observation after the end of HCV treatment. Time from SVR to reinfection was estimated using Kaplan–Meier analyses.
Results: 338 patients cured from HCV were included. Men represent 77% and women 23% of the sample; mean age was 46 years; major risk factor for HCV infection was IDU for 275 (82%) patients, sexual transmission for 30 (9%) and the remaining patients were infected from tattoos (n = 9), transfusion/contaminated liquid (n = 11), sharing of non IDU material (n = 4) or were from endemic regions (n = 5). Patients were followed up (FU) for a median of 2.7 years after the end of treatment (IQR, 1.7–4.8), for a total of 1175 person-years of FU. 316 (94%) patients remained persistently negative, while 22 (6%) became reinfected during FU period with an overall reinfection rate of 1.7/100py [95% CI 1.07–2.58]. Median time from cure to reinfection was estimated at 14.7 years (95% CI 13.6–15.7). Cumulative incidence of seroconversion within 2 years of SVR was 4% (9/210) and 11% (10/88) within 5 years of SVR. Following adjustment for past or present drug use, the incidence rate of HCV reinfection was 0.43/100py [95% CI 0.02–0.11] for non drug users; 1.90/100py [95% CI 1.13–3.14] for past IDU and 3.60/100py [95% CI 1.44–7.39] for present IDU.
Conclusions: HEPVIRAC shows a relative low risk of HCV reinfection after successful treatment. Although the rate of HCV reinfection is higher in IDU than non IDU, it remains much lower than the overall incidence rate of the first HCV infection in drug users in Montreal.

P1251
HEPATITIS B AND C: KNOWLEDGE, ATTITUDES AND PREVENTIVE PRACTICES OF THE GREEK GENERAL ADULT POPULATION. PRELIMINARY RESULTS OF THE GREEK NATIONAL HEALTH EXAMINATION SURVEY "HPROLIPSIS"

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Background and Aims: Awareness, standpoints and practices of the general population are of major importance for planning prevention and treatment programmes. We aimed to investigate knowledge level, attitudes and preventive measures for hepatitis B (HBV) and C viruses (HCV) infection in the general Greek adult population (≥18 years).

Methods: Data from the ongoing Greek study Hprolipsis, the first Health Examination Survey of HBV, HCV and HIV in the general population and in vulnerable populations (immigrants and Roma), were analyzed. Multistage stratified random sampling based on 2011 census was applied to select the sample. A standardized questionnaire was administered by trained interviewers. Questionnaire data collected during the period March-August 2014 were analyzed.
Results: In total 2074 subjects were included in this analysis, 923/1151 male/female, median age 56.2 years (IQR: 40.4–68.8). Only 29.6% and 19.5% of the participants had a good knowledge level of HBV and HCV, respectively. These low percentages were mainly attributed to the high levels of misunderstandings regarding transmission modes. 34.1% and 37.2% believed that HBV and HCV are transmitted by daily social contact and 45.7% and 48.3% by insect bites, respectively. Results from multivariable analysis revealed a trend for lower knowledge scores with older age, lower education level and lower family income. 44.2% did not usually use condom. More frequent condom use was associated with age <40 years and higher educational level. The majority (58.4%) of the population was not aware of HBV vaccine. Awareness of HBV vaccine was higher in females (43.9% vs 36.8% for males, p = 0.011). The majority of the participants were not aware of the possibility of treatment (HBV: 67.3%, HCV: 75.3%). Testing history for HBV and HCV has been reported by 25% and 18.8% of subjects, respectively. Age >40 years and lower educational level were associated with lack of HBV/HCV testing history. Among those tested, positive results for HBV and HCV infections were reported by 5.9% and 1.5%, respectively.
Conclusions: There are significant knowledge gaps in the Greek general population regarding modes of transmission, preventive measures and treatment availability for both HBV and HCV. There is an urgent need for large scale awareness activities to fill the gap of knowledge and to increase population engagement in preventive measures.

P1252
PREVALENCE OF HBV SEROLOGICAL MARKER IN GENERAL HEALTHY POPULATION

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Background and Aims: Hepatitis B virus (HBV) infection is a serious global public health problem affecting billions of people globally. In China, HbsAg, was used to determine the prevalence HBV instead of HBcAb. Furthermore, HBcAb was in ELISA assays diluted 1:30 in ELISA assays. The lack of information of HBcAb seroprevalence without diilution among the general healthy population is an obstacle for evaluating the prevalence of HBV. Therefore, this population based serological survey was conducted in Sichuan province, China, where no large epidemiological data was available.
Methods: 7603 healthy subjects were selected in west China. The subjects' age ranged from 6 to100 years old. Serum samples were tested for HBcAb, HBeAg, HBeAb, HBsAg and its antibody. Screening tests were carried out by Elecsys assays
Results: The prevalence of HBsAg and HBcAb in our district was before 0.57% and 57.6%, respectively. The prevalence of HBcAb were more seen in males (P < 0.05) and in 40–50 age groups. We found 3194 of 3511 HBcAb negative individuals were HBsAb positive alone. What's more, the distribution of HBsAb positive rate was associated with the age distribution, in other words, the young people has less exposure to HBV suggesting the HBV vaccine plan helps to control HBV spread.
Conclusions: Approximately 57.6% of general healthy population has prior exposure to HBV and less than 1% is HBsAg carriers. HBV vaccine plan should be emphasized and successful in China.

P1253
SIMULTANEOUS HIV–HBV–HCV POINT-OF-CARE TESTS IMPROVE THE SCREENING OUTCOMES

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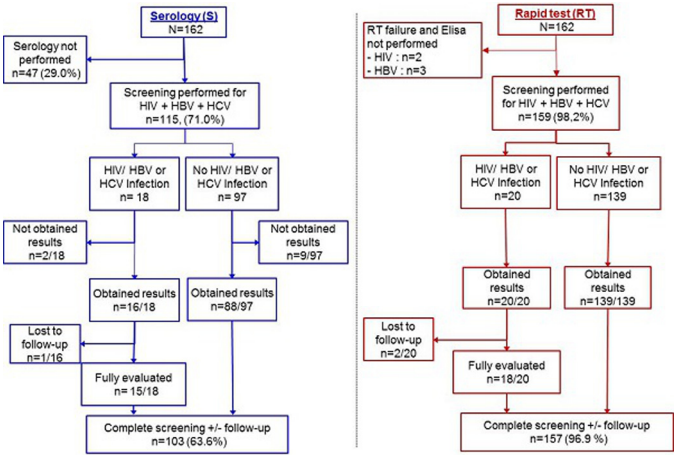
Background and Aims: In France, more than 55% of people infected with HBV, 45% of those infected with HCV, and 17% of HIV-positive persons are unaware of their status. The study objective was to evaluate the usefulness of simultaneous HIV-, HBV- and HCV-rapid tests on improving infection awareness in a population of predominately immigrants.
Methods: The OptiScreen III study was a randomized, prospective study in an urban clinic for persons without healthcare coverage ("Medecins du Monde"). Participants were randomized 1:1 to receive: standard HIV, HBV and HCV serology (S), where participants received a prescription to perform veinopuncture at an outside laboratory and then return to the clinic to obtain their results, or combined rapid tests (RT) (VIKIA® HIV, VIKIA® HBsAg and Oraquick® HCV), where participants received testing at the clinic and could opt to obtain results 30mn latter. Study endpoints were the proportion obtaining test results and, among those with positive serology for any test, seeking further care within four months.
Results: Of the 162 participants randomized to receive standard, only 104 (64.2%) returned to retrieve their test result. In comparison, 159/162 (98.2%) of those randomized to the RT arm obtained their results ($p < 0.001$). Thirty-eight (11.7%) tested positive for HIV (n = 7), HBV (n = 23), or HCV (n = 8) and no co- or tri-infection was observed. There were no significant difference between RT and S arms ($p = 0.7$). In the RT and S arms respectively, 20/20 (100%) and 16/18 (88.9%) of infected participants received their screening result ($p = 0.2$) and 18/20 (90.0%) and 15/18 (83.3%) received specialized care ($p = 0.7$). Overall, 157 (96.9%) in the RT arm were appropriately linked to screening and further linked to care, if necessary, versus 103 (63.6%) in the S arm (p < 0.001).
Conclusions: The simultaneous use of HIV-, HBV- and HCV-rapid tests improved the proportion screened. However, seeking further care might be influenced by the individual's motivation.



P1254

HEPATITIS C SPONTANEOUS CLEARANCE MAY INDUCE PARTIAL PROTECTION AGAINST REINFECTION AMONG PERSONS WHO INJECT DRUGS: RESULTS FROM A COHORT STUDY

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Background and Aims: In Montréal, Canada, HCV transmission has remained high over the past decade among people who inject drugs (PWID). However, HCV reinfection has not been estimated. A related key question is whether the nature of the initial immune response during primary infection confers partial immunity against reinfection, and whether this potential effect may differ according to the clearance mechanism, i.e., spontaneous vs. treatment induced. Our aims were to estimate the incidence rate of HCV reinfection among PWID in Montreal, and to examine the association between HCV reinfection and the clearance mechanism, adjusted for associated behavioural factors.
Methods: PWID were recruited into a prospective cohort study between 2004 and 2013. HCV reinfection rate was estimated among anti-HCV-positive/HCV-RNA-negative participants well characterised for previous HCV clearance mechanism (spontaneous vs. treatment-induced). At each semi-annual visit, data were collected by validated interviewer-administered questionnaire and blood samples were tested for HCV-RNA to detect new infections. Time-to-event methods were used to estimate incidence rates (IR). Time-updated Cox regression models were conducted to examine factors associated with HCV incidence.
Results: Of 226 participants included in analyses [76% males, mean age 38.7 years (SD: 9.9)], 128 (57%) had previously cleared their infection spontaneously. Overall, 33 participants became reinfected with HCV [IR = 4.8 per 100 p-year (95% CI: 3.36, 6.65)]. Reinfection IR were 4.07 per 100 p-year (95% CI: 2.5, 6.2) among the 128 spontaneous resolvers, and 6.33 (95% CI: 3.60, 10.37) among the 98 participants who cleared their infection through treatment, respectively. In multivariate analysis, participants who cleared their infection through treatment were 2.5 times more likely to become reinfected compared to spontaneous resolvers, after adjusting for risk behaviours. Prescription opioid injection and cocaine injection were the main behavioural characteristics associated with reinfection (Table 1).

Table 1. Factors associated with HCV reinfection

Variable	Crude HR (95% CI)	Adjusted HR (95% CI)
HCV initial clearance		
Spontaneous clearance (ref)	1	
Clearance through treatment	1.79 (0.9, 3.7)	2.49 (1.2, 5.3)
Less than 30 years of age	1.70 (0.8, 3.6)	1.34 (0.6, 3.1)
Gender (female vs. male)	0.88 (0.4, 2.2)	0.78 (0.3, 2.0)
Incarceration past 3 months	0.94 (0.3, 3.1)	0.95 (0.3, 3.3)
Injection cocaine past month	1.77 (0.8, 3.7)	1.93 (1.0, 4.2)
Injection heroin past month	1.19 (0.5, 2.6)	0.91 (0.4, 2.1)
Injection prescription opioid past month	3.52 (1.7, 7.1)	4.05 (1.9, 8.7)
Sharing syringes or injection paraphernalia past 6 months	1.67 (0.8, 3.5)	1.48 (0.7, 3.3)

Conclusions: Our results support the hypothesis that the initial immune response induced during HCV spontaneous clearance is likely associated with long-lived, highly efficient memory immune responses that can partially protect against re-infection, relative to treatment-induced clearance. Injection prescription opioid and injection cocaine are the main drivers of HCV reinfection among PWID, calling for targeted prevention strategies.

P1255

ACCESSING HEPATITIS C TESTING: WHO, WHAT, WHERE, AND WHEN?

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Background and Aims: Clinical pathways for persons with Hepatitis C (HCV) are important to quantify, to understand and manage factors that influence the uptake of healthcare interventions, continuity in care and outcomes in persons with HCV, with the overall aim to reduce onward transmission. We describe the distribution of persons testing for HCV and diagnosis rates by health care services.
Methods: Data were extracted from the sentinel surveillance programme of blood born viruses which include all testing undertaken by a network of 24 sentinel laboratories within England irrespective of test result. Data for persons who were first reported to the sentinel surveillance programme during 2013 were analysed. Data were excluded if persons were aged less than one year, or the samples were dried blood spot, oral fluid, reference testing, and testing from hospitals referring all samples.
Results: During 2013, 188, 364 persons were tested for HCV, with three out of five tests conducted in primary care settings. Overall, the highest proportion of HCV testing was conducted within GP surgeries (29.9% of all tests) followed by GUM clinics (18.4%), with the lowest proportion within drug dependency services and HIV clinics both 0.8%. The distribution of persons testing positive for anti-HCV by service type was similar to that of tests with 70% of persons with a positive result testing within primary care settings. A third of positive tests were among those requested at GP surgeries, and 17.1% from GUM clinics, with little change in the distribution of tests over the past three years. Despite differences in the proportion of tests and diagnoses between primary and secondary care settings the diagnosis rate was similar with 2.2% and 1.3% respectively. While, drug dependency services represented the lowest proportion of tests in 2013, the diagnosis rate was the highest at 10.1%, followed by prisons with 9.4%. Diagnosis rates for both these testing services have fallen over the past 3 years, falling from 18.0% to 10.1% for drug dependency units and 11.9% to 9.4% in prisons.
Conclusions: Overall a higher proportion of persons are accessing testing in primary care settings, in particular GP surgeries and GUM clinics suggesting persons are presenting earlier within the course of their infection, and a suitable setting through which undiagnosed infections can be detected. However, positivity rates are still associated with services accessed by high risk individuals such as drug users.

P1256

THE GLOBAL AND REGIONAL BURDEN OF LIVER DISEASE, 2013

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Background and Aims: The Global Burden of Disease Study 2013 (GBD 2013) is a collaboration of more than 1000 researchers in over 100 countries, which provides comprehensive morbidity and mortality estimates for 291 diseases and injuries for the global population between 1990 and 2013.
Methods: Estimates for the annual number of deaths attributable to incident viral hepatitis, and for cirrhosis and liver cancer

attributable to hepatitis B, hepatitis C, alcohol and other causes were obtained for the years 1990 to 2013 by region and for the global population. These estimates were compared with all cause mortality to explore changing trends in the relative burden of liver diseases over time.
Results: Global deaths related to liver disease increased from 1.5 million in 1990 to 2.1 million in 2013, with those attributable to viral hepatitis increasing from 895,000 to 1.4 million over this period. Deaths from liver cancer due to hepatitis B infection increased by 50%, while liver cancer deaths causes by hepatitis C were estimated to have more than tripled between 1990 and 2013. Cirrhosis deaths for all causes increased steadily over time. Deaths due to incident hepatitis A and hepatitis B decreased, particularly after 2000, but those from hepatitis E were estimated to remain stable.
Conclusions: Chronic liver disease, particularly due to viral hepatitis, is responsible for a significant and increasing proportion of human deaths and ranks among the leading contributors to global mortality. The ability of GBD 2013 and future GBD studies to systematically compare trends in liver disease burden between populations and over time allows for local prioritisation of policy and public health interventions, and the capacity to monitor the impact of these responses into the future.

P1257
EARLY IDENTIFICATION OF PATIENTS AT RISK FOR CHRONIC HEPATITIS B AND C IN ARNHEM REGION, THE NETHERLANDS

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Background and Aims: Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are often asymptomatic and therefore diagnosed late at a stage when liver damage such as fibrosis, cirrhosis or hepatocellular carcinoma (HCC) has already occurred. The challenge is to identify patients early. Between 2009 and 2014 we conducted five screening projects in Arnhem region to identify patients at risk who are not yet aware of their HBV- and HCV infection. All participants were offered free screening on HBV and/or HCV.
Methods: In order to reach our target group we used different approaches. We organized educational meetings about HBV and HCV, and developed brochures, posters, video's and websites (community based). In two screening projects the target group received a personal invitation and a flyer with information on the educational meetings offering free onsite testing for HBV and HCV (personal invitation). We approached volunteers from the respective target groups and trained them to be 'ambassadors' of the project (key persons). We mainly focused on first generation migrants (FGM) from seven middle and high endemic countries, but also did a HCV screening project among (ex) intravenous drug users (IDU) who had never been tested before. We also performed a hepatitis awareness campaign 'Heptember', offering free HBV and HCV testing for all persons considering themselves to belong to the risk group for HBV of HCV.
Results: We found that only screening projects combining community approach, use of key persons with personal invitation could achieve high participation rates up to 36%. In total we tested 2710 participants and identified 68 persons with hepatitis B (positive HBsAg) and nine with hepatitis C (positive HCV-RNA) (2.5% and 0.3% respectively). Clinical evaluation revealed that four patients had liver cirrhosis, two with HBV and two with HCV.
Conclusions: Personal invitation of FGM, seems to be very promising. In the future it should be integrated into daily practice of primary health care. To target the risk groups for HCV infection, such as (ex) IDU, seems to be more complicated. Many (ex) IDU are not in care and are difficult to reach. Therefore, also community base programs like "Heptember" (revealing two ex IDU with chronic hepatitis C) seem to be an effective method to identify these patients at risk and need to be continued.

P1258
PREVALENCE OF SIGNIFICANT HEPATIC FIBROSIS AND CIRRHOSIS ASSESSED BY THE NAFLD FIBROSIS SCORE IN PATIENTS ATTENDING THE DIABETIC CLINIC

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Background and Aims: Non-alcohol fatty liver disease (NAFLD) is common in diabetic patients with metabolic syndrome. NAFLD fibrosis score (NFS) is a validated non-invasive scoring system that helps to identify patients with significant fibrosis and cirrhosis. This score can help to determine high risk patients warranting hepatology assessment and hence, applying NFS to routine diabetic care appears useful. The aim of this study is to apply the NFS to patients attending the diabetic clinic to assess the prevalence of significant fibrosis and cirrhosis.
Methods: We retrospectively examined all patients attending the diabetic clinic from March to June 2014 and included patients with type II diabetes and impaired glucose tolerance. Patients with type 1 diabetes and gestational diabetes were excluded. Data were obtained from laboratory database and electronic diabetic patient record (Cellma).
Results: Of the 521 patients screened, only 29.4% (153) of patients with complete laboratory data were studied. In our cohort of 153 patients, the median age was 63 (IQR 56.0, 71.5) years and 64.1% (98) of patients were male. More than half of our patients (56.2%; n = 86) had a BMI >30 kg/m^2. Of these, 14.4% (22) of patients had a BMI >40 kg/m^2, 14.4% (22) had a BMI of 35–40 kg/m^2 and 27.5% (42) had a BMI of 30–35 kg/m^2. The median HBa1c reading was 53 (44, 61) mmol/mol. Using the NAFLD fibrosis score, almost a quarter of our patients (24.2%, n = 37) had significant fibrosis with a median score of 1.169 (0.898, 1.563). The majority of our diabetic patients (66.0%, n = 101) had an indeterminate score of −0.224 (−0.744, 0.302). Only 15 (9.8%) patients had no significant fibrosis with a score of −1.820 (−2.500, −1.550).
Conclusions: Using the NFS in routine diabetic care, the prevalence of NAFLD with significant fibrosis was estimated to be close to a quarter of all patients. Only a small proportion of patients were assessed to have no significant fibrosis.

P1259
HEPATITIS B IN PREGNANCY IN UKRAINE: WASTED CHANCE

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Background and Aims: Limited data regarding the prevalence of HBV in countries of the former USSR suggest that it is much more common than in Western Europe (3–14.4%). Since perinatal transmission is associated with a higher rate of viral chronicity, vertical transmission could be an important contributor to the epidemic of HBV in countries with high prevalence.
Methods: To assess the prevalence of HBV in a subset of young female patients and practices of care for pregnant woman with HBV, we performed a retrospective review of delivery records at 2 centers serving an area with a population of 105,000 in Central

Ukraine and 250,000 in Western Ukaine. Comparison was made to EASL Guidelines for HBV in pregnancy. All pregnant women in Ukraine are offered free prenatal care and HBsAg testing is a part of routine screening. The number of deliveries, presence of HBsAg, attitudes toward HBV infant vaccination among women post partum, and treatment offered were studied.

Results: The results are outlined in the table. HBsAg was detected in 0.72% of pregnant women. None had additional testing for HBeAg or viral load. None were receiving anti-viral therapy and none were offered consultation for HBV post-partum. Although many children were vaccinated for HBV, those at risk for vertical transmission did not receive HBIG even though it is manufactured in Ukraine. Attitudes toward vaccination are positive in the majority of these mothers, however, distrust and worry about potential side effects precluded achievement of 100% vaccination of newborns for HBV. Internal turmoil and trouble with vaccine purchase and distribution may have led to the recent observed decrease in vaccination rates.

Conclusions: HBsAg was detected in 0.72% of pregnant Ukranian women. No treatment was offered to these mothers with HBV. Prophylaxis of vertical transmission of HBV was not carried out effectively, however, vaccine is available and was given to most newborns. Education of health care providers regarding EASL Guidelines, available treatment options and prevention of vertical transmission with HBIG would greatly improve care for these mothers and infants. Despite constitutional guarantees of access to health care in Ukraine, limited resources were allocated to these patients with HBV. With the new government and development of closer ties to the European Union it is hoped that such change will occur.

Year	No. of births	% HBsAg+	% vaccinated
2008	3907	0.87%	79.5%
2009	3967	0.91%	74.4%
2010	3827	0.89%	66.4%
2011	3715	0.62%	47.8%
2012	3828	0.57%	61.7%
2013	3821	0.47%	14.1%

P1260
PERCEPTIONS OF VIRAL HEPATITIS TESTING IN MIGRANT COMMUNITIES AND THE PRIMARY CARE PHYSICIANS THAT SERVE THEM

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Background and Aims: Migrant groups are disproportionately affected by hepatitis B and C (CVH). Effective community testing must be culturally-appropriate, and ideally include informed testing in primary care. General Practitioners (GPs) offer a direct and effective route for community testing, but participation is thought to be poor, with little study into the awareness, understanding and perception of GPs towards CVH case-finding. We conducted focus group sessions among members of the Nepali community; a new and unique community to the UK, as well as GPs to study and contrast the awareness and perception of both groups to CVH and CVH testing.

Methods: We conducted 4 focus group sessions in the Nepali community; divided by age (greater/less than 30 years) and sex. We explored the understanding and perception of liver disease and viral hepatitis, including developing new testing strategies. We also conducted focus group sessions among GPs from 2 large General Practices in South East England that serve this growing Nepali community. We explored the understanding and perception of viral hepatitis case-finding, including recent national testing guidance.

Results: 32 members of the Nepali community took part in focus group sessions. A strong extrinsic association was seen related to most concepts of liver disease, including spiritual causes/cures and food pollution. Younger groups associated a greater degree of stigmatisation towards liver disease from alcohol or prostitution. However all groups wanted to engage with liver disease education and testing, and universally so via primary care services.

9 GPs of varying seniority, from military and civilian backgrounds took part in the focus group session. Overall CVH testing was viewed as a low priority, with one senior GP expressing that they had not seen a single case in the past 20 years. No GP was aware of national case-finding guidance, despite its recent release and community focus. Migrant testing was not offered by any GP, with the feeling that this would be viewed as "racial profiling" by members of the migrant community.

Conclusions: Members of the Nepali community expressed universal interest in engaging with primary care to learn more about liver disease and testing. However, the GPs in the same area viewed CVH case-finding as a low priority, with little awareness of national or international guidance for migrant testing. Stigmatisation was raised by some younger members of the migrant focus group, but was almost a universal concern to GPs.

P1261
SPECIFIC PATTERNS OF DRUG CO-USE INCREASE RISK FOR HEPATITIS C VIRUS INFECTION IN PEOPLE WHO INJECT DRUGS

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Background and Aims: Prescription opioid injection (PO) and poly-substance use were shown to be associated with hepatitis C virus (HCV) transmission among people who inject drugs (PWID). Little is known, however, about the role of specific drug use patterns in the risk of HCV infection. The aim of this study was to examine the association between HCV seroincidence and use of specific drugs and their combination among PWID, with a focus on PO injection.

Methods: Data from a prospective cohort in Montreal (HEPCO) were used. PWID who were HCV and human immunodeficiency virus (HIV)-seronegative at baseline and completed ≥1 visit between 2004 and 2013 were included. At each visit, data were collected by validated interviewer-administered questionnaire and blood sample was tested for HCV-RNA and antibodies to detect new infections. Kaplan–Meier survival analysis and time-varying Cox regression models were conducted.

Results: Of the 356 participants (81.2% males; mean age: 34.7 years), 123 participants (34.6%) reported PO injection. Overall, 115 seroconverted to HCV (incidence rate: 14.51/100 person-years [95% CI: 12.0, 17.4]). In univariate analyses, use of the following drugs was associated with HCV incidence: injecting PO (crude hazard ratio [cHR]: 3.14 [95% CI: 2.2, 4.6]), cocaine (cHR: 4.04 [95% CI: 2.6, 6.4]), or heroin (cHR: 1.63 [95% CI: 1.1, 2.4]); using non-injection tranquilizers (cHR: 1.75 [95% CI: 1.2, 2.5]), or smoking crack/cocaine (cHR: 1.54 [95% CI: 1.1, 2.2]). Co-use patterns involving PO injection (compared to no use of either drugs) associated with HCV included: PO with tranquilizers (cHR: 4.12 [95% CI: 2.5, 6.7]), PO with cocaine injection (cHR: 10.86 [95% CI: 5.8, 20.3]), PO with crack/cocaine smoking (cHR: 4.14 [95% CI: 2.5, 6.7]). In multivariate analysis, PO injection was independently associated with HCV infection compared to non PO injection. Further, PWID who co-used

all three drugs with PO injection had the highest risk of HCV acquisition (Table 1).

Table 1. Unadjusted and covariate-adjusted associations

Variable	Crude HR (95% CI)	Adjusted HR (95% CI)
Drug use past month		
No injection PO (ref)	1	1
Injection PO + 0–2 of the 3 drugs [a]	2.54 (1.7, 3.9)	1.94 (1.2, 3.0)
Injection PO + 3 drugs	6.01 (3.6, 10.1)	4.94 (2.9, 8.5)
Age (5-year increment)	0.91 (0.8, 1.0)	0.97 (0.9, 1.1)
Male	0.97 (0.6, 1.5)	0.79 (0.5, 1.3)
Unstable housing conditions past month	2.13 (1.5, 3.1)	1.96 (1.3, 2.9)
Incarceration past 3 months	1.82 (1.2, 2.9)	1.90 (1.2, 3.0)
Sharing syringes or injection equipment past 6 months	2.10 (1.4, 3.0)	1.83 (1.2, 2.7)

HR, hazard ratio.
[a] Injection cocaine, crack/cocaine smoking, non-injection tranquilizers.

Conclusions: Our results suggest that PO injection and its co-use with cocaine injection, non-injection tranquilizers, or smoked crack/cocaine increases the risk of HCV infection for PWID. In addition, our data point to the importance of polysubstance use as major driver of HCV transmission for PO injectors. These findings suggest that comprehensive HCV prevention strategies should address polysubstance abuse. Specifically, PO injectors using multiple drugs should be identified as high priority targets for interventions.

P1262

AN ACCURATE SCORE FOR THE PREDICTION OF SUSTAINED VIRAL RESPONSE UNDER PROTEASE INHIBITOR-BASED TRIPLE THERAPY IN CIRRHOTIC PATIENTS: THE CUPIC ALGORITHM – THE ANRS CO20 CUPIC STUDY

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Background and Aims: Antiviral therapy is highly required in patients with chronic hepatitis C (CHC)-induced cirrhosis, to decrease liver-related complications or death. New drugs allow for high rates of sustained viral response (SVR) in genotype 1 CHC with very few side effects, but these treatments are not yet widely available. As a consequence the standard of care for cirrhotic patients in many countries currently remains the triple therapy using protease inhibitors (PI) Boceprevir (BOC) or Telaprevir (TEL). We aimed to develop an algorithm for the prediction of SVR after PI-based triple therapy in cirrhotic patients.

Methods: 484 patients from the ANRS CO20 CUPIC cohort were included and randomly divided in a derivation and a validation sets. All patients had genotype 1 CHC-induced cirrhosis and were treated by triple therapy using BOC or TEL according to standard of care.

Results: 52.1% of patients achieved SVR. In the derivation set, independent predictors of SVR among data collected at day0 were: treatment history (previous response to dual therapy by PEG-interferon + ribavirin), TEL treatment, gammaGT, platelets, and viral load. These 5 easy-to-collect variables were combined in the d0-score to predict SVR before treatment initiation. When data collected at w4 of triple therapy were introduced in the model, independent predictors of SVR were: treatment history, gammaGT, platelets, and w4 viral load. These 4 variables were combined in the w4-score to predict SVR since 4 weeks of triple therapy. Thresholds for very low (≤10%) or very high (≥90%) chance of SVR were calculated for both scores. The sequential use of the d0-score and then the w4-score, called the CUPIC algorithm, predicted SVR in the validation population with 85% accuracy in 54% of patients. The 46% remaining patients had 50% SVR rate (grey zone). As "treatment history" is not available in naïve patients, we used the same methodology to develop 2 others scores without this variable (independent predictors: genotype 1a, IL28B, gammaGT, platelets, TEL treatment, d0 and w4 viral load). The sequential use of these 2 scores predicted SVR with 84% accuracy in 48% of patients of the validation population.

Conclusions: By using simple variables, the CUPIC algorithm predicts PI-based triple therapy success or failure before or since week4 of treatment. This algorithm will help physicians to balance between treating now patients having CHC-induced cirrhosis with PI-based triple therapy or waiting for new drugs.

P1263

GLOBAL ESTIMATE OF HCV INFECTION IN THE PEDIATRIC AND ADOLESCENT POPULATION

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Background and Aims: A number of studies have reported global estimates of HCV infections, but little attention has been paid to HCV infection among pediatric and adolescent (Ped) populations aged 15 years old or younger. The aim of this study was to develop a global estimate of anti-HCV and viremic prevalence in this population using extrapolations from published studies.

Methods: A comprehensive literature search was conducted to identify studies reporting HCV infections that were representative of the general Ped population. Analyses in high risk populations (e.g., patients on dialysis, chemotherapy, and hemophiliacs) were excluded. Data were available in 24 countries (Egypt, Pakistan, Ethiopia, Cameroon, Thailand, Tunisia, …). A ratio of HCV infection in Ped to adult population was calculated for each country and the results were grouped by country income classification. These ratios were then applied to HCV prevalence estimates in adults in all countries in the same income classification. Given the high spontaneous clearance in the Ped populations, a viremic rate of 50–75% was applied to the anti-HCV estimates based on published studies in the Ped population.

Results: The Ped to Adult anti-HCV prevalence ratio was 4% (3%>5%, 90% uncertainty interval) in high income countries, 21% (19%>25%) in upper middle income countries, 28% (20%>43%) in lower middle income countries and 54% (38%>106%) in low income courtiers. Globally, 13.2 (11.5–21.2) million Peds are estimated to be anti-HCV positive, corresponding to an anti-HCV prevalence of 0.7% (0.2–1.3%). There are 6.6 (6.1–11.6) million viremic infections corresponding to a viremic prevalence of 0.4% (0.1–0.8%). Viremic prevalence was lowest in high income countries at 0.3% (0.03–0.5%) and highest in low income countries at 0.6% (0.1–1.0%). Over 85% of all HCV infections are in low income and low middle income

countries with Nigeria, Egypt, Pakistan, India, China, Ethiopia and the Republic of Congo accounting for over 50% of all HCV infections in the Ped population.

Conclusions: HCV infection in the pediatric and adolescent populations is highest in low income and low middle income countries where access to care is already limited. Clinical and epidemiological studies in this population are needed as soon as possible to tackle HCV infection. This would ensure early access of the Ped population to care and inclusion in clinical trials with novel safer therapies.

P1264

ADDRESSING THE HCV PUBLIC HEALTH BURDEN: SURVEILLANCE OF SOFOSBUVIR'S IMPACT ON EU5 MARKETS AND HOW, IF AT ALL, TREATMENT RATES, DRUG SHARES AND PRESCRIBING HABITS CHANGED

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Background and Aims: Despite the HCV therapeutic market evolving at speed, access constraints across the EU5 (France, Germany, Italy, Spain, UK) mean HCV remains a major public health burden. Focusing on genotype 1 (G1) HCV patients we explore – at a country level – HCV treatment rates, uptake of sofosbuvir, and specific differences between patients treated with sofosbuvir-based regimens (i.e. + RBV / + IFN / + other DAAs) and those untreated.

Methods: Ipsos Healthcare's HCV Therapy Monitor, running since 2006 in the EU5, reports on >240 physicians per quarter across the EU5. Physicians provide patient demographic, disease and treatment data on HCV patients seen within each study period.

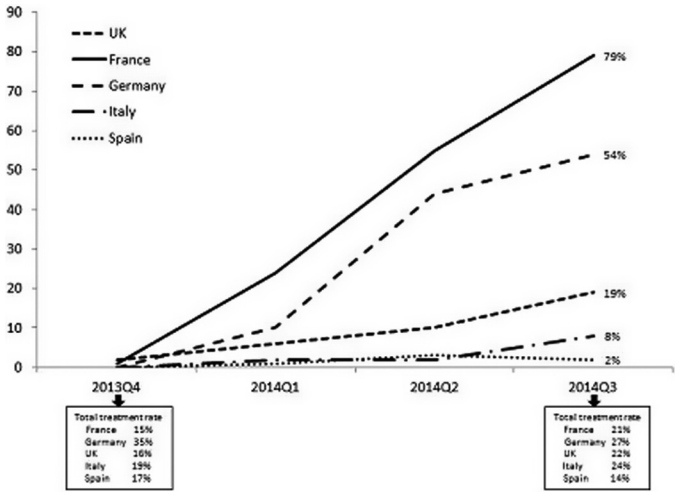

Figure: Percentage of genotype 1 patients treated with a sofosvubir-containing regimen over time.

Results: Overall, treatment of HCV patients in the EU5 is low – below 30%. Germany has the highest proportion of treated patients and Spain the lowest (2014 Q3 data) (Figure 1). Rates are static, apart from in France where significant increases are seen (2014 Q3 data). The EMA approval of sofosbuvir in January 2014 led to accelerated uptake of the drug in France and Germany – reaching 79% and 54%, respectively, of all treated G1 patients (Figure 1). This was not replicated in the other EU5 markets, where local price negotiations meant sofosbuvir only became widely available in October 2014; uptake among treated patients in the UK is below 20% and, in Spain and Italy, below 10%. 98% of UK physicians (and comparable numbers in Spain and Italy) cited cost and access as barriers to sofosbuvir use. Looking at characteristics of sofosbuvir-treated patients, those in France are more likely to have F3 or F4 (compensated) liver damage than those in Germany. In both countries, physicians treat fewer substance abusers and patients with concomitant conditions.

Conclusions: As our results show, the rapid evolution of HCV treatment is not matched by increasing treatment rates. The approval of curative sofosbuvir-based regimens was highly anticipated; however, price and access, alongside long-term warehousing, have clearly impeded higher proportions of patients being treated – especially in UK, Spain and Italy where drugs take longer to be approved. Country-specific disparities are also seen in physicians' selection of patients for treatment; it appears they are having to prioritise treatment for certain patient types. With G1 patients representing the largest proportion of EU5 HCV patients, timely access to new regimens and a uniform consensus to treatment is essential to curb the spread of HCV.

P1265

ESTIMATING THE EPIDEMIOLOGIC AND ECONOMIC IMPACT OF DIFFERENT TREATMENT RATES FOR HEPATITIS C VIRUS (HCV) IN EGYPT

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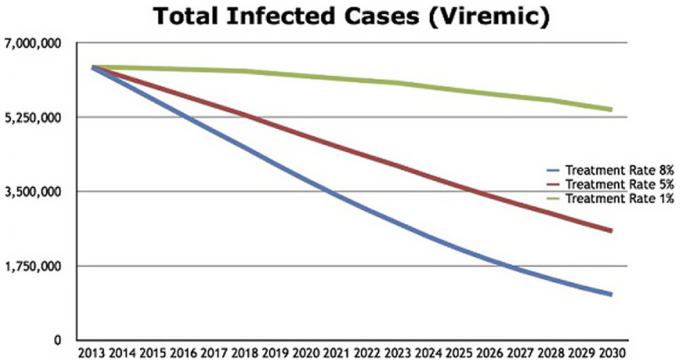
Background and Aims: The burden of HCV infection in Egypt is one of the highest globally (est viremic cases at 6.5 mill). New promising Hep C antiviral agents are more efficacious, but more costly, posing challenging policy questions related to rates of treatment and budget allocations. This analysis attempts to quantify the epidemiologic and economic impact of different treatment rates in Egypt.

Methods: The analysis was based on a 17-year Markov state transition cohort with annual cycle lengths. At baseline, viremic patients were divided into 5-year age increments. Disease severity at baseline was modelled on the basis of data in the US [i], subsequently calibrated to data severity for Egypt [ii]. In the model, patients would then progress through the various HCV stages from F0 to F3, based on international transition probabilities [iii] and were also at general population risk of death. Following the state of F3, patients could progress to hepatocellular carcinoma or cirrhosis. Following cirrhosis to other progressive stages up to hepatocellular carcinoma and liver related death. Patients could be cured spontaneously in state F0 or with antiviral therapy in any state from F0 to cirrhosis at a cure rate of 92% in stages F0–F3 and a cure rate of 80% in the stage of cirrhosis. Cured patients would transition to the mortality risk of the age-matched general population. In each cycle, new incident patients were introduced at a rate of approximately 128,000 or 2%.



Results: The model indicates significant gains using the new antivirals when following a treatment rate of 8% (450k viremic cases/year) relative to a policy of 1% (65k cases/yr) or 5% (300k cases/yr). By 2030 viremic cases would go down from

5.5 to 2.5 to 1 million cases in the 1%, 5% and 8% treatment rate scenarios. Liver related deaths from 41,000 to 24,000 to 14,000 cases by 2030 respectively. Total annual costs would start at $1.3 billion on the first year of treatment in the 8% treatment rate pattern but decreases reaching an annual budget of $580 million by 2030. Respectively 5% and 1% rates would start at $900 million and $550 million to go down to $600 million and $500 million by 2030.

Conclusions: Treating patterns using the new antiviral Hep C agents from 300k/yr to 450k/yr would have a substantial clinical benefit. It would cost the society (government and private) more, in the short run, but eventually they would start running out of patients, so costs would go down. With 450k/year, you can get to around 1% prevalence by 2030.

P1266

HUMAN ALBUMIN USE IN CIRRHOTIC PATIENTS IN FRANCE: RESULTS OF THE NATIONAL "ALBU-LIVE" SURVEY

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Background and Aims: Use of human albumin (Alb) in the management of complications of cirrhosis has increased in recent years. Three indications are validated: therapeutic paracenteses (TP), spontaneous bacterial peritonitis (SBP) and hepatorenal syndrome type 1 (HRS); in these 3 indications EASL recommendations have been published [*J Hepatol.* 2010 Sep; 53(3)]. Aims of this survey were to assess prescription practices of French hepato-gastroenterologists in validated indications and to seek for differences according to experience and work location of practitioners (P).

Methods: All P working in Hepato-Gastroenterology units of general and university hospitals (GH and UH) and private clinics were individually contacted. A pre-established questionnaire evaluated: (1) use of albumin in validated indications and (2) prescription for other clinical situations for which albumin use is not validated. The survey was conducted between April 1 and August 10, 2014.

Results: 451 answers were obtained and analysed. The mean age of P was 40 [20–67] years. Location of exercise was UH (47.7%), GH (45.8%), liberal activity (3.3%). 56.7% were senior-P, 6.5% attached physicians, 12.4% assistants and 24.4% residents.

TP: 99.6% of P compensate for paracentesis. 87.8% of P compensate only with Alb; 12% use other volume expanders. When Alb is used, 84.6% use a fixed dose: 20 g for 3 liters in 2/3 of cases. The infusion begins during (52.6%) or after (32.3%) the paracentesis.

SBP: 94% use albumin infusion concomitantly with antibiotic treatment. The EASL-protocol (1.5 g/kg D1–1 g/kg D3) is respected by 56.2%, more often by senior-UH than by senior-GH (60.3% vs 42.1%; $p = 0.015$).

HRS: 66.5% of P use Alb infusion to confirm the diagnosis of HRS; senior-SH more often than senior-PH (81.2% vs 57.8%; $p = 0.0006$). For treatment, 84% of P use Alb concomitantly with vasopressor treatment but the dose and the duration are very heterogeneous.

Other clinical situations: 23.5% use Alb for severe bacterial infections (except SBP), 47.9% for severe hyponatremia, 43.9% for severe hypoalbumemia and 65.9% after thoracocentesis for hydrothorax. 19.1% of P use Alb in other circumstances.

Conclusions: In this large French survey, albumin prescription is in accordance with EASL-recommendations only for TP. The EASL-schedule in SBP is followed only by 56%. The use of albumin for diagnosis and treatment of HRS is not adapted with EASL-guidelines and very heterogeneous. Finally, Alb in cirrhotic patients is beyond the scope of the approved indications in many cases.

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RETRIEVAL OF PATIENTS WITH CHRONIC HEPATITIS B AND C BY INTENSIFYING REGIONAL COLLABORATION, ARNHEM REGION, THE NETHERLANDS

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Background and Aims: Excellent treatment is available for chronic hepatitis B virus (HBV) and hepatitis C virus (HCV). However, many patients once diagnosed for HBV of HCV have been lost to follow-up in primary care and/or hospital care. The challenge is to retrieve these patients and bring them back into care.

Methods: We organized two meetings at the hepatitis centre of Rijnstate hospital with participation of the municipal health service (MHS), general practitioners (GP), centre for drug addicts, health service in prisons, midwives in primary care and two microbiological laboratories in the region. In the first meeting we formulated problems, in the second meeting we made a definite plan to retrieve patients who have been lost to follow-up.

Results: 1. In the hospital we checked hospital records of all patients been seen with HBV since 2007 at the department infectious diseases. We found that 72 out of 242 patients were lost to follow

up or had no advised control of ALAT in primary care. All these patients are being traced.
2. In a pilot study of two general primary care practices (2300 patients each) 30 patients (21 with HBV and nine with HCV) were retrieved by a laboratory search 2000–2014. We identified ten patients in need for re-evaluation and if necessary referral to our hepatitis centre.
3. All pregnant women are screened for HBV in primary care, with the main purpose of prevention of mother to child transmission. These women are often lost to follow-up after delivery, without adequate evaluation for potential need for therapy. We established a plan for retrieval of these women with a major role for the laboratories and the municipal health service.
4. All patients with a positive HBsAg test are automatically being reported by the laboratories to the MHS. The MHS compared their registration list with all patients being in specialist care in the hospital and found that 64 out of 230 (28%) referred patients never arrived at the hospital. These patients are being traced.
Conclusions: Regional retrieval of patients with HBV and HCV infection is possible with close collaboration of all regional health providers for hepatitis. At the same time the organisation of regional hepatitis care can be optimized, in order to avoid unnecessary loss to follow-up of patients in the future. However, it is time consuming and will need extra budgeting for extending the project to other regions in the Netherlands.

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THE CORRELATION BETWEEN DEPRESSION AND CHRONIC HEPATIC CONDITIONS AS MEASURED BY THE PHQ-9 DEPRESSION ASSESSMENT ACCOUNTING FOR SOCIAL CHARACTERISTICS

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Background and Aims: Most insurance payors in the United States are requiring the results of a standardized depression assessment prior to initiating treatments for chronic liver diseases. Existing research shows that depression rates among patient populations who are experiencing viral hepatitis are more than twice as high as the general US population. What is less known are the social factors including class, gender, and race/ethnicity impact the relationship between depression and viral hepatitis. Our aim is to review the utility of knowing patient depression scores in the treatment of liver diseases as a whole-body approach to medicine. We ask: how do social factors statistically moderate the correlation between depression and viral hepatitis and other chronic liver conditions? Does knowing the social circumstances of patients contribute to the treatment design for patients with any chronic hepatic condition?
Methods: We assessed 274 consecutive patients with one or more chronic hepatic conditions including viral hepatitis, liver cancer, non-alcoholic steatohepatitis. The patients have an average depression score of 11.5±8.6 out of a possible 27 points. The data contains 112 female patients and 159 male patients with a mean age of 53.2±13.9. We further measured SES indicators including zip code (proxy measure for income), race/ethnicity to measure if these factors impacted the relationship between depression and chronic hepatic conditions.
Results: Based on logistic regression models, the findings show patients with high depression scores (>15) are 35% more likely to be Hispanic compared to other ethnic groups, 61.7% more likely to be women than men and 64.7% more likely to be from impoverished communities when compared to more affluent communities. Patients with 3 or more comorbidities experience depression at nearly double the rates of patients with only one chronic hepatic condition.
Conclusions: While research has consistently shown that patients with chronic liver disease are likely to have higher than average depression rates, our analysis of social factors shows: depression rates are higher for (1) Hispanics; (2) women; (3) patients who live in impoverished communities; and (4) patients with comorbidities. Future research should consider socioeconomic and demographic characteristics and control of comorbidities as they impact depression and progression of chronic liver disease.

P1269
"YOU'RE BETTER OFF WAITING": KNOWLEDGE AND AWARENESS OF HEPATITIS C DIRECT-ACTING ANTIVIRALS IN A COHORT OF PEOPLE WHO INJECT DRUGS

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Background and Aims: People who inject drugs (PWID) are the group at greatest risk of hepatitis C (HCV) infection. They are also the group most likely to transmit the infection, and should be a priority population for treatment as a public health initiative. Although few currently receive treatment, the advent of new direct-acting antiviral (DAA) treatment provides hope for increased uptake of therapy. The Burnet Institute and St Vincent's Hospital in Melbourne Australia will commence recruitment for the Hepatitis C *Treatment and Prevention* (TAP) Study in December 2014, The TAP Study will examine the feasibility of treating PWID in the community using directly acting antiviral therapy (DAAs) and evaluate a network-based approach to treatment and its impact on HCV prevalence and incidence. To inform recruitment of the TAP Study we interviewed PWIDs to better understand their knowledge of the DAAs and identify their perceptions on the best methods to include them in HCV care and treatment.
Methods: Participants were recruited from the SuperMIX cohort comprising over 700 PWID followed since 2008 and included eligible TAP study participants. In-depth interviews were conducted with twenty-three HCV RNA positive participants. Interviews were transcribed, coded, and then analysed for emerging themes and similarities between participants. General descriptions and critical interpretation of themes were generated and selective quotes extracted verbatim to best illustrate the critical themes. The analysis of the interviews was shaped by the knowledge of the data and the existing literature.
Results: Whilst most PWID had a basic understanding of HCV, for many it was limited. The majority of participants had limited knowledge of HCV treatment, with most being unaware that DAAs would soon be available. Once the researcher explained that DAAs would have high efficacy and a low side effect profile the majority were very interested in undergoing treatment and also having their injecting partners treated for their HCV.
Conclusions: This study found that limited knowledge and understanding of HCV restricted participants ability to make a well-informed decisions about their HCV management. However when presented with accurate information, most were enthusiastic about accessing DAA treatment. It is important that we engage and work with PWID if we are to successfully implement HCV treatment programs that benefit both the individual infected with HCV and prevent the ongoing transmission of the virus.

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HEPATITIS C AND B TESTING AMONG PEOPLE LIVING WITH HIV IN ENGLAND

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Background and Aims: The British HIV Association guidelines for the management of hepatitis viruses in HIV-positive adults, recommends testing for hepatitis, B and C at HIV diagnosis, and annually thereafter. The sentinel surveillance of blood-borne viruses collects laboratory data irrespective of test result; providing information on the population undergoing HIV and hepatitis testing at 16 sentinel laboratories.

Methods: Demographic information and HBV and HCV testing histories for persons newly diagnosed with HIV between 2008 and 2013 were extracted from the laboratory information systems of 16 sentinel laboratories in England. Where self reported ethnicity was unavailable, it was assigned using name analysis software. Duplicate records, reference testing and individuals aged <16 years at HIV diagnosis were excluded. Characteristics of individuals tested and testing positive for HIV, HBV and HCV were described. Data were managed in MS Access and analysed in R (CRAN).

Results: In total, 1,936,792 persons were tested for HIV across all settings, of whom 17,962 (0.9%) tested positive. Two thirds of all HIV positive persons were male (12,228/17,691) and where known, three quarters were white ethnic origin (75.9%; 6426/8463). Overall, 75.8% (13,412) of HIV positive persons were tested for anti-HCV, of whom 5.9% (797) tested positive. Of anti-HCV positive persons, 86.3% (688) were tested for HCV-RNA, of whom 75.9% (522) were positive indicating an active infection.

HCV genotype information was available for 73.3% (383) of all HCV-RNA positive persons. Genotype 1 infections were the most prevalent (61.8%; 237), followed by genotype 3, genotype 4 and genotype 2, representing 23.7%, 12.0% and 2.5%, respectively. HCV genotype distribution did not vary significantly by gender ($\chi^2 = 1.64$; $p = 0.57$), or by age group, but did vary by ethnic group ($\chi^2 = 19.84$; $p = 0.02$).

Overall 72.4% (13,011) of HIV positive persons were tested for HBsAg, of whom 4.5% (591) tested positive. Of these, 62% (372) were tested for HBV DNA, of whom 84.9% (315) tested positive.

Conclusions: Approximately three-quarters of persons newly diagnosed with HIV were tested for HCV and HBV. Of these 522 and 591 persons were identified as being HIV/HCV and HIV/HBV co-infected, respectively. These findings suggest that while screening rates for HCV and HBV are high, further work is necessary to monitor and improve testing rates, and reduce the significant contribution of HCV and HBV towards hepatic morbidity and mortality in HIV infected individuals.

P1271

HOW SHOULD SCALE UP OF HCV ANTIVIRAL TREATMENT BE PRIORITIZED? A COST-EFFECTIVENESS ANALYSIS INCLUDING INDIVIDUAL AND POPULATION PREVENTION BENEFITS

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Background and Aims: New HCV direct-acting antivirals (DAAs) will dramatically improve cure rates but are associated with high costs. European guidelines recommend prioritizing DAAs for severe liver disease for individual benefit, but earlier treatment of those at risk of transmission such as people who inject drugs (PWID) may be more cost-effective. We determine the most cost-effective HCV treatment prioritization strategy by disease stage and risk status.

Methods: We use a previously developed dynamic HCV transmission and disease progression cost-effectiveness model to compare prioritization of HCV treatment using interferon-free DAAs. We evaluate providing treatment at earlier disease stages (mild or moderate fibrosis) and by risk status (PWID or ex-PWID) compared to delaying treatment until compensated cirrhosis. We assume 12 week treatment duration (£3200/wk) and 90–95% SVR, depending on disease stage. We explore settings with populations with chronic HCV prevalence among PWID at 20, 40, or 60%. We perform a probabilistic cost-utility analysis estimating long-term costs (in £UK=€1.25) and outcomes as quality adjusted life-years gained (QALYs) discounted 3.5%/yr. We rank strategies by calculating Net Monetary Benefit (NMB) = (mean incremental QALYs × Willingness to pay [WTP] threshold) − mean incremental costs. We use a WTP threshold of £30,000 (~€37,500) per QALY gained. Highest NMB has highest rank.

Results: After treating people with cirrhosis, in populations with 20% or 40% chronic prevalence among People Who Inject Drugs (PWID), which is consistent with most sites in Europe, it is more cost-effective to prioritize treatment to PWID at earlier disease stages because of substantial prevention benefits (Table 1) – rather than delay treatment. Only in populations with very high (60%) chronic HCV prevalence among PWID is it more cost-effective to prioritize DAAs to non-PWID with moderate disease (Table 1).

Table 1. Net monetary benefit of treating People Who Inject Drugs (PWID) or non-injectors with mild or moderate disease compared to delaying treatment until development of severe disease

Rank	Group	Net monetary benefit (£30,000 WTP)
20% baseline chronic prevalence among PWID		
1	PWID, mild	£13,267,319
2	PWID, moderate	£7,365,353
3	Ex/non-PWID, moderate	£2,796,417
4	Ex/non PWID, mild	£919,001
40% baseline chronic prevalence among PWID		
1	PWID, moderate	£5,894,771
2	PWID, mild	£5,581,928
3	Ex/non-PWID, moderate	£2,796,417
4	Ex/non PWID, mild	£919,001
60% baseline chronic prevalence among PWID		
1	PWID, mild	£2,796,417
2	PWID, moderate	£1,562,238
3	Ex/non PWID, mild	£919,998
4	Ex/non-PWID, moderate	£919,001

Conclusions: In many European settings it will be more cost effective to treat People Who Inject Drugs at earlier disease stages rather than delaying treatment. Guidance on treatment allocation and prioritisation when scaling up HCV treatment needs to take account of disease stage and potential prevention benefit.

P1272

A COMPARATIVE STUDY OF PATIENT SATISFACTION WITH LIVER DISEASE CARE IN THE UNITED STATES AND URBAN AND RURAL CHINA

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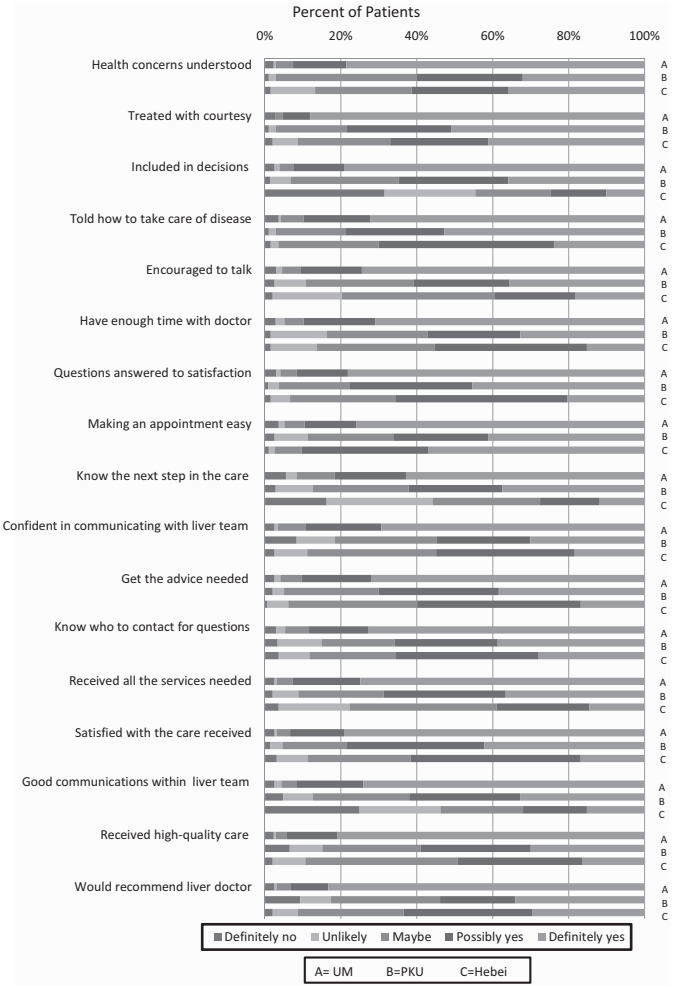
Background and Aims: Patient satisfaction is an important measure of quality of care and may be different for different

diseases and different clinical settings. There is no validated tool to measure patient satisfaction with liver disease care. Aims of this study were: (1) To validate an 18-item survey for patient satisfaction with cancer care in patients with liver disease. (2) To compare satisfaction with care among patients attending liver clinics in the United States (U.S.) and in urban and rural China and to determine factors associated with satisfaction with liver disease care.

Methods: Established adult patients with liver disease seen in the liver clinics at University of Michigan (UM), Peking University (PKU) and Hebei clinic were invited to participate in this study. The "Patient Satisfaction with Cancer Care" questionnaire (Jean-Pierre P, et al. Cancer. 2011; 117: 854–61) was tested for validity in patients with liver disease and for internal consistency. Results from UM, PKU and Hebei patients were compared.



Results: A total of 990 patients were analyzed between 6/2014 and 9/2014, 395 UM, 398 PKU and 197 Hebei. Median age was 53 years and 59.8% were men. Most common liver disease was HCV at UM (43.5%) and HBV at PKU (67.8%) and Hebei (49.2%). 40.3% of patients at UM, 43.2% at PKU and 15.2% at Hebei had been seen at the liver clinics for >5 years. Exploratory factor analysis revealed a 1-dimensional measure with 17 of 18 items forming a coherent set explaining 73% of the variance in patient satisfaction at UM, 62% at PKU and 68% in Hebei. Reliability assessment revealed high internal consistency (0.97 at UM, 0.94 at PKU and 0.95 in Hebei). Mean total satisfaction score (maximum = 85) of patients at UM was higher than at PKU (78 vs. 66, $P < 0.001$); which in turn was higher than in Hebei (66 vs. 60, $P < 0.001$). Univariate analyses revealed that site, age, education, type of liver disease, liver disease knowledge, and duration of follow up at liver clinic were associated with satisfaction with liver disease care. Multivariate logistic regression analysis revealed that site was the only factor associated with satisfaction with liver disease care.

Conclusions: The Patient Satisfaction with Cancer Care survey is a valid tool for assessing satisfaction with liver disease care. Patient satisfaction with liver disease care in the U.S. is better than in urban China, which in turn is better than in rural China. Understanding these differences may improve patient satisfaction and outcomes.

P1273
ACUTE PORTAL VEIN THROMBOSIS: CLINICAL FEATURES, DIAGNOSIS AND OUTCOMES AFTER 5 YEARS OF FOLLOW-UP

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Background and Aims: Acute portal vein thrombosis (APVT) is a rare thrombotic obstruction of extrahepatic/intrahepatic portal venous system, associated with local and systemic risk factors. The clinical features APVT are poorly defined in the literature. The proportion that progress to chronic PVT and the influences of various treatments are unknown. The aim was to summarize the clinical features of APVT in a Portuguese reference center as well as their evolution over five years of follow-up.

Methods: A total of 5 APVT patients admitted in our hospital from 2008 to 2009 were analyzed retrospectively.

Results: APVT was diagnosed in 1 female and 4 males, with a mean age of 47 years old. Most patients (n = 4) presented with abdominal pain and tenderness. Only one patient had cirrhosis by the time of diagnosis. A hereditary thrombofilia was detected in 2 cases, an intra-abdominal infection in 1, a mieloproliferative disease in 1, and nocturnal paroxistic hemoglobinuria in another one. Diagnosis was confirmed by angio-CT in 3 patients (60%) and MRI in another 3 (40%). None of the patients underwent thrombolysis nor thrombectomy. Four patients (80%) were anticoagulated after diagnosis (warfarin). The one patient with cirrhosis was not anticoagulated because of previous hemorrhagic events, and died soon after. The mean duration time of anticoagulation was 39 months, and was effective in half of the patients (n = 4). Intestinal infarction was the solo complication reported in one patient. No other patient died within the period of follow-up.

Conclusions: Our data reveals to be similar to that found in the literature, showing the value of following the available recommendations regarding diagnosis and therapeutics. It also confirms that anticoagulation therapy is the treatment of choice in this rare disease, with real impact on long time survival.

P1274
SUPPORTIVE AND PALLIATIVE CARE IN ADVANCED LIVER DISEASE: PATIENTS' NEEDS AND PRIORITIES

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Background and Aims: The number of people dying with advanced liver disease is rising rapidly, yet little is known about the support needs and priorities of patients and their families as they approach the last year of life. Palliative care services are increasingly recognising the needs of non-cancer patients, but liver disease remains relatively neglected. This study aimed to illuminate the needs and experiences of people with advanced liver disease and those of their lay and professional carers to inform their appropriate supportive and palliative care. Specifically, it explored the dynamic physical, psychosocial, existential and information needs of patients and their lay and professional carers, and their use and perceptions of health, social and voluntary services.

Methods: Multi-perspective, serial in-depth interviews. Patients with advanced liver disease of different aetiologies were recruited in hospital. Patients and their lay carers were interviewed up to 3 times over one year. Case-linked professionals were interviewed once. Interviews were recorded, transcribed and analysed using grounded theory techniques and NVivo 9 software.
Results: 15 patients and their lay and professional carers were recruited, and 53 interviews conducted. Uncertainty emerged as the central factor dominating experiences across all dimensions of need, at all stages of the illness, and for all participants: patients, lay carers and professionals. This uncertainty related to the nature of the illness, the unpredictability of physical deterioration and prognosis, poor communication and information-sharing, and complexities of care. Current care arrangements were a poor fit with the high levels of physical and psychosocial need identified. The pervasive uncertainty meant that planning for deterioration and death was largely neglected.
Conclusions: Uncertainty lies at the heart of the experiences of patients, lay and professional carers. As a result, the needs of this patient group are currently poorly met from diagnosis to bereavement. Uncertainty makes it especially important to plan ahead to help people live and, in due course, die as well as possible. Professionals must acknowledge and actively manage this uncertainty to improve liver patients' quality of life and facilitate their appropriate and timely supportive and palliative care.

P1275
STIGMA AND DISCRIMINATION IN VIRAL HEPATITIS – THE VOICE OF THE PATIENT

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Background and Aims: Stigma and discrimination affect the quality of life of those infected with viral hepatitis. Few studies have evaluated the circumstances and the degree to which stigma and discrimination are present, but these studies are based on the view of health professionals. In the present study, we have tried to listen to the voice of the patient in order to find out from the own infected in what contexts, and with what intensity stigma and discrimination affect their quality of life.
Methods: A questionnaire with 12 items was used to evaluate how the carrier of hepatitis feels stigmatized and discriminated against. 1,217 people infected with hepatitis B or C in Europe and Americas were interviewed, by the electronic system SurveyMonkey®. Participation was voluntary, anonymous, and each participant has accepted a term of consent.
Results: The results show that 49.6% of those infected have suffered some kind of discrimination. Of the 94.1% who reported the infection to the family, 24.6% said that relatives started to avoid physical contact. Of the 73.7% who reported their condition to friends, 46.9% claims to have suffered discrimination and 23.8% were no longer invited to social events. Of the 57.4% who reported their condition to their partners, 33.3% had their relationships affected. 42.7% of infected had their sexual life affected. Of the 46.1% who reported their condition to work colleagues, 10.1% lost their jobs. Their self-image was affected in 55.8% of the cases and 41.4% felt ashamed of their condition. Although the survey indicates that 70% of health professionals attended carriers properly, 24.6% of those professionals have maintained a certain distance from the patient and 6.9% denied care to people infected with hepatitis. 21.2% also claim to have suffered some kind of discrimination at the dentists, 10.8% when they were taking tests at a laboratory and 17.1% claim to have been discriminated against in the beauty segment (manicure / pedicure). Thus, 38.7% of respondents said that the population has some degree of rejection in the face of illness and that 33.5% of people do not show solidarity and understanding.
Conclusions: The results are extremely important because they allow us to identify the situations and population segments where discriminatory behavior and stigma are more frequent, enabling the design of strategies and action plans to fight these adverse situations that much impair the quality of life of those infected with viral hepatitis.

P1276
VITAMIN D SUPPLEMENTATION FOR CHRONIC LIVER DISEASE – A COCHRANE HEPATO-BILIARY GROUP SYSTEMATIC REVIEW

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Background and Aims: Vitamin D deficiency seems common in patients with chronic liver disease. Our aim was to assess the effects of vitamin D supplementation in patients with chronic liver disease.
Methods: We searched databases until Feb 2014 for randomised clinical trials that compared vitamin D3 (cholecalciferol), vitamin D2 (ergocalciferol), 1α-hydroxyvitamin D (alfacalcidol), 25-hydroxyvitamin D (calcidiol), or 1,25-dihydroxyvitamin D (calcitriol) supplementation at any dose, duration, and route of administration versus placebo or no intervention in patients with any chronic liver disease. We conducted meta-analyses, using intention-to treat. We used risk ratios (RR) and risk difference (RD) for dichotomous outcomes, and mean difference for continuous outcomes, all with 95% confidence intervals (CI).
Results: Six randomised trials with 325 participants provided data for analyses. All trials were with high risk of bias. Vitamin D supplementation had no significant effect on all-cause mortality (RD 0.01, 95% CI −0.02 to 0.04; 6 trials, 325 participants) (vitamin D3 800 to 2,000 IU; vitamin D2 14,286 to 21,429 IU; calcitriol 0.25 to 1 μg daily) or on liver-related mortality (RR 1.62, 95% CI 0.08 to 34.7; 1 trial, 18 participants). Vitamin D3 had no significant effect on rapid virological response (RR 0.24, 95% CI 0.01 to 4.58), but showed significant beneficial effect on early (RR 0.09, 95% CI 0.03 to 0.25) and sustained virological response (RR 0.23, 95% CI 0.11 to 0.52) in the two trials with 122 chronic hepatitis C patients. Calcitriol had no significant effect on acute cellular rejection in liver transplant recipients (RR 0.33, 95% CI 0.04 to 2.62; 1 trial, 75 participants). Vitamin D2 had no significant effect on liver function tests in patients with alcoholic cirrhosis (1 trial, 18 participants).
Conclusions: The lack of sufficient number of trials and sample size, low quality and paucity of data prevents us from drawing firm conclusions regarding effects of vitamin D supplementation on the meta-analysed outcomes in chronic liver disease patients. Vitamin D supplements have no statistically significant effects on survival, liver-related mortality, adverse events, the number of patients with rapid virological response, or acute cellular rejection in liver transplant patients, but data were sparse. Vitamin D3 might improve early and sustained virological response in chronic hepatitis C patients. Further trials with low risks of systematic and random errors seem warranted.

P1277

SCREENING FOR VIRAL HEPATITIS AMONG MIGRANTS IN THE EU: WHAT LESSONS CAN WE LEARN FROM POOR RESPONSE TO A PILOT TRIAL USING GP REGISTERS?

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Background and Aims: Effective treatments for chronic infection with hepatitis B and C virus (HBV, HCV) are available. However, many infected individuals remain undiagnosed and cannot benefit from treatment. In particular migrants may be difficult-to-reach due to language and cultural barriers. The HEPscreen Project aimed to test different ways of screening migrants for viral hepatitis. Here we evaluate the benefits of a pilot study of general practitioner (GP) based testing for viral hepatitis in East London.
Methods: Registers of two GP practices in East London were screened for migrants at risk of viral hepatitis. African, South Asian and Turkish patients were selected as 'at-risk' ethnicities. Patients were included if they were aged 18 and over, had registered with the GP in the last 5 years and did not have known infection with HBV or HCV. Identified patients were sent invitation letters with information sheets in their main language and asked to attend the practice for testing. Half of the patients were additionally invited for HIV testing to investigate the influence on uptake. Subsequently, two focus groups were held with African and Turkish individuals who had not taken part in the pilot. The setup of the pilot and possible reasons for non-attendance were discussed.
Results: A total of 560 patients were invited, 200 African, 170 South Asian and 190 Turkish. Overall uptake was poor, with only 6 African, 3 South Asian and 4 Turkish patients attending for testing (2.3%). No infected patients were found and the pilot was stopped prematurely due to insufficient uptake. This prevented a formal analysis of the benefit or harm of including HIV testing in the program but of note 9 patients who were invited for hepatitis only testing attended compared to 4 patients for HIV and hepatitis testing. The focus groups highlighted a number of general and specific barriers to testing: relationship with GPs, attitude towards research studies, low levels of awareness of hepatitis B and C, sensitivity around their community being targeted for health screening, fear, and tone and content of the invitation letter and information sheet. The inclusion of HIV testing in the invitation was expected to reduce uptake.
Conclusions: GP registers in the UK can identify migrants at risk of viral hepatitis but a number of barriers need to be recognised and overcome if this is to be a cost effective intervention.

P1278

EFFECTS OF "CHRONIC HEPATITIS PATIENTS INFORMATION AND MORALE MEETINGS" ON PATIENT COMPLIANCE AND TREATMENT SUCCESS

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Background and Aims: Chronic hepatitis causes patients and their relatives to worry about their health and future, to feel isolated from the environment, to develop feelings of abandonment and affects their psychological state negatively. These negative factors prevent proper diagnosis and treatment. That's why, we organize "hepatitis patients information and morale meetings" to inform the patients and their relatives about hepatitis, to correct misconceptions and to improve their relationship with treatment team since 2007 in Mersin. These meetings are arranged twice a year in spring and autumn. About 150 patients with chronic hepatitis and their relatives are invited to each meeting. Meetings are informal and start at 10:30 with brunch on Sunday. All participants are encouraged to actively participate and join the conversation. To evaluate the efficiency of meetings and level of knowledge before and after the meeting quantitatively, we provided a survey with patients at two meetings of last year.
Methods: Patients were asked to fill a questionnaire before and after the meetin
Results: A total of 205 interviews were completed. Half of the participants were male, mean age was 47. 41% of participants were primary school graduates, 43% of them attended the meeting before. 59% of them had chronic HBV, 39% of them had chronic HCV. 59% of them had comorbidity. 13% of participants stated that they were excluded by their relatives after illness. 29% of participants were afraid of having a chronic disease and liver cirrhosis. 25% of them were worried about having a difficult to treat, contagious disease. 95% of the patients stated that they felt better after meetings, and negative psychological effect of disease decreased after meetings (5.9/10 to 7.1/10). They became more optimistic, they understood that it was a treatable disease and there is a group of doctors who care about them. They felt informed and their level of information rised from 6.3/10 to 8.6/10 and this increment was statistically significant ($p = 0.001$). Some of the patients stated that they used their drug regularly after the meeting. 99% of patients and their relatives wanted the meetings to continue and recommended to other patients.
Conclusions: These meetings had positive effects on their psychological state and level of knowledge about disease. This would increase their faith and their desire to continue treatment

P1279

MORTALITY, PREMATURE DEATH AND DIRECT COSTS ASSOCIATED WITH LIVER DISEASE IN PORTUGAL

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Background and Aims: Health professionals have recognized liver disease as a common cause of morbidity and mortality in Portugal. However, few studies have estimated the socioeconomic impact of these disorders. Our study aims to determine the number of hospital discharges and admissions, mortality, premature death and costs associated with liver disease from the perspective of the National Health Service in Portugal.
Methods: A descriptive, retrospective analysis of data from 97 hospitals between 2000 and 2008 and mortality data for 2011 collected from the Portuguese National Institute of Statistics (Instituto Nacional de Estatística [INE]). The 9th and 10th revisions of the International Classification of Diseases were used to establish diagnoses. Demographic data, average length of stay (ALOS), in-patient mortality and direct costs associated with hospital admissions and liver transplant were compared for the most common liver diseases. Based on national mortality data from people aged 70 years-old and younger (INE 2011), we estimated years of potential life lost index (YPLL) and compared liver disease with other conditions.
Results: The annual mean number of discharges for liver disease was 11,503 between 2000 and 2008. Most cases of liver disease were diagnosed in men (70.4%) and the prevalence of liver disease peaked in patients aged from 20 to 64 years (60.7%). Alcoholic cirrhosis was the most frequent liver-disease diagnosis (30.0%). The mean ALOS was 10 days and the mortality rate was 13%. Daily direct hospital costs ranged from €450 per patient for chronic hepatitis B infection to €1163 for chronic hepatitis C infection. In 2008, costs associated with liver transplantation were €15,259,275. The mortality rate for all liver diseases was 13 per 100,000 inhabitants in Portugal. Liver disease imposed the third greatest burden based on the YPLL in 2011. This YPLL result exceed these

attributed to certain conditions such as cerebrovascular diseases and some malignant neoplasms.
Conclusions: Liver disease imposes a considerable social and economic burden on Portugal. Our results suggest that educational, legislative and therapeutic interventions to prevent morbidity, mortality and premature death from liver disease are urgently required to minimise the economic and clinical burdens.

P1280
THE DYNAMICS IN GENERAL AND LIVER DISEASE SPECIFIC ASPECTS OF QUALITY OF LIFE AMONG PATIENTS WITH CHRONIC LIVER DISEASE IN YUNNAN, CHINA

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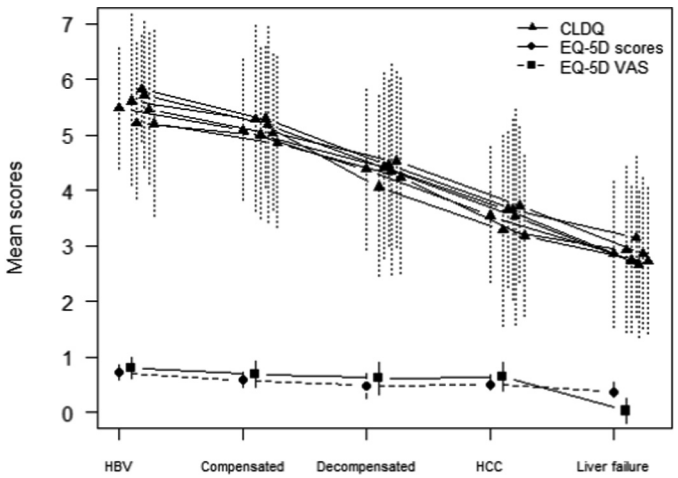
Background and Aims: *Background:* Patients with chronic liver diseases (CLD) may compromise their health related quality of life (HRQoL). Hepatitis B virus (HBV) infection has long been the leading cause of CLD including liver cancer and cirrhosis. Knowledge on different symptom profiles of CLD help to develop comprehensive treatment and patient care plans.
Objective: To access the facets of HRQoL in chronic liver diseases throughout the spectrum of severity of the disease.
Methods: A cross-sectional study was conducted in the First Affiliated Hospital of Kunming Medical University in Yunnan province of China. Both out- and inpatients undergoing treatment protocols for different HBV related liver disease states were consecutively collected from December 2012 to June 2013. ANOVA was used to compare the mean scores of EQ-5D and chronic liver disease questionnaire (CLDQ) among 5 disease groups. The relationship between demographic variables predicting global CLDQ scores and the domains of CLDQ was analysed.
Results: A total of 1040 patients including 520 without complication, 91 with compensated cirrhosis, 198 with decompensated cirrhosis, 131 with HCC and 100 with liver failure were recruited. All domains of CLDQ, the means of EQ-5D value and EQ VAS exhibited significant decline with worsening of disease severity from uncomplicated HBV to liver failure. The multivariate regression demonstrated the reduction of mean scores of CLDQ domain at advanced stage. Patients with liver failure and HCC had more HRQoL impairment than other disease states. No effect of patient gender was found. Patient age was associated with 'fatigue' and 'worry' domains ($p = 0.006$; $p = 0.004$) but not with other domains and global scores of CLDQ and ED-5D.
Conclusions: The HRQoL in chronic hepatitis B patients has been greatly affected by disease states. The success of care for HBV-related diseases should consider not only in the outcomes of treatment strategies but also in improving patient's wellbeing.



P1281
HEPATOLOGICAL MANIFESTATION OF DENGUE VIRAL FEVER – A CLINICO BIOCHEMICAL STUDY IN ADULT PATIENTS

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Background and Aims: Dengue is a common arboviral infection of tropics and sub tropics transmitted globally and is becoming a major international health concern. Adult data of liver involvement in dengue fever are scarce. The aim of this study was to determine the frequency and pattern of hepatic manifestation in Dengue fever.
Methods: A retrospective study of 48 patients of serologically confirmed dengue fever from two centres was done. All the patients had liver involvement either in the form of elevated transaminases, jaundice, increased prothombin time and or ascites. These patients were admitted during the recent outbreaks of 2013 and 2014.
Results: Of the 48 patients 37 were male and 11 female (M:F = 3.4:1). Mean age: 32.6 (range 16–60). Mean platelet count was 45.98×10^9/L (range: $10–160 \times 10^9$). Fever was present in all. Pain abdomen in 26 (54.2%), vomiting in 18 (37.5%). Melena was seen in 10 (20.8%) and 2 patients had encephalopathy. Clinical jaundice was observed in 7 (14.5%), ascites in 6 (12.5%), hepatomegaly in 28 (58.3%). 10 (20.8%) patients had hyperbilirubinemia (Ser Bil >2). AST and ALT was elevated in all the patients. Mean AST and ALT levels were 402.6 and 225.6 IU/ml respectively. ALT was two to five times elevated in 22 (45.8%) and more than five times elevated in 12 (25%). AST elevation was more than ALT in 40 (83.3%) patients. AST:ALT ratio more than 2 was observed in 17 (35.4%) patients. Serum Alkaline Phosphatase level was elevated in 21 (43.8%). Prothrombin time more than 3 s was observed in 9 (18.8%). Hypoalbuminemia (Ser Alb <3) was present in 19 (39.6%). Mean Ser albumin – 3.02 g/dl. Arterial ammonia was elevated in the 2 patients who developed encephalopathy. Ultrasonography revealed ascites in 20 patients which was mostly minimal to mild in quantity, hepatomegaly in 18 and splenomegaly in 6 patients. 5 (10.4%) patients developed Acute liver failure. Two patients died due to acute liver failure.
Conclusions: Dengue hepatitis manifest as fever, abdominal pain, hepatomegaly, thrombocytopenia, transaminase rise mainly preferential elevation of AST, with or without jaundice and minimal to mild ascites in majority. Acute liver failure may occur. Level of thrombocytopenia is not associated with disease progression.

P1282
WHAT IS NEEDED TO CONTROL HEPATITIS C (HCV) IN DIFFERENT EPIDEMIOLOGICAL SETTINGS?

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Background and Aims: Hepatitis C is a mojor cause of global mortality. There are scalable tools to combat HCV, including harm reduction interventions for people who inject drug (PWID), procedures to minimise medical risk, and new highly effective HCV treatments. We use modelling to consider how combination interventions could bring HCV mortality and incidence down to low levels in three epidemic scenarios
Methods: A model of HCV transmission was developed considering HCV transmission amongst PWID and due to unsafe medical procedures. The model was parameterised for three epidemiological scenarios, with all having a PWID chronic HCV prevalence of 60% and overall HCV chronic prevalence of either 1, 3 or 5%. The impact of different interventions were modelled incrementally,

firstly harm reduction (HR – opiate substitution therapy and needle and syringe provision, each at 50% coverage and 50% efficacy), then a 75% reduction in transmission risk due to unsafe medical procedures (MR), and lastly three HCV treatment scenarios of either all cirrhotics treated each year, or treatment of 10% of all infected per yr but either distributed to just non-PWID or all infecteds

Results: For the 1, 3 or 5% overall HCV chronic prevalence epidemic scenarios, PWID contributed 90, 32 or 20% of new incident HCV infections but a smaller proportion of prevalent infections. Results suggest that HR can have substantial impact (50%) on preventing onwards transmission in settings driven by PWID, but will have smaller impact (10%) in settings with substantial iatrogenic transmission. In these settings, reductions in iatrogenic transmission are crucial for reducing incidence. HCV treatment is required to quickly reduce HCV mortality, and can reduce HCV incidence if targeted to sub-groups driving transmission. In PWID-driven epidemics, this means targeting HCV treatment towards PWIDs to reduce incidence but non-PWID to reduce mortality as few PWID are cirrhotic. In non-PWID driven epidemics, treatment of cirrhotics will immediately prevent HCV mortality and may reduce HCV incidence. Treatment of PWID will have a smaller impact on transmission in these scenarios because they contribute less to transmission

Conclusions: A halving in new infections could be achieved rapidly (10 yrs) based on scaled-up harm reduction to 50% of PWID and 75% reduced risk of medical exposure. A halving in deaths by 2030 could also be achieved if 10% of the chronically infected population are treated annually, with greater impact if cirrhotics are prioritized

P1283
HEALTH RELATED QUALITY OF LIFE IN CIRRHOTIC PATIENTS IN AUSTRIA

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Background and Aims: The majority of patients with chronic liver disease are living with the disease rather than dying from it. A number of systemic symptoms have been recognized that can occur at any point in the natural history of the disease and which can be associated with functional impairment and reduced quality of life.

Aim of the study: Health related impairment of quality of life in an Austrian group of patients with cirrhosis

Methods: 50 consecutive unselected hospitalized patients (43 men and 7 women; age 58.26+11.39 years) with a diagnosis of advanced chronic liver disease (Child–Pugh Score 9.62+1.92). A sociodemographic questionnaire included marital state, religion, life style variables, level of education and present working status. For the evaluatation of the impairment of quality of life the german version of the Chronic Liver Disease Questionnaire (CLDQ-D), and the Hospital Anxiety and Depression Score (HADS-D) was used as well as the questionnaire in "Resources in Sexuality and Partnership" (R-S-P). For correlations between severity of liver disease and impairment of different qualities of life, Spearman's rank correlation coefficients were calculated.

Results: See the table.

Conclusions: All HRQL domain scores were declined in our cirrhotic patients, as compared to published data of healthy persons. These findings are similar to results found in different European and US populations. Since sexuality and partnership is not included in the classical quality of life scores, but is known to be relevant in cirrhotics, we performed in addition a questionnaire concerning sexuality and partnership. All domain scores were as well declined compared to published normal values, with the largest difference in tenderness and love. An influence of the severity of liver disease on quality of life, could be seen in total CLQD, and in the subset of fatigue, but not in the anxiety and depression score, and not in the sexuality and partnership questionnaire.

Questionnaire	Score, mean±SD	Correlation with Child–Pugh score
CLDQ-D		
Total	4.77±1.13	p = 0.037
Abdominal symptoms	5.02±1.71	p = 0.066
Fatigue	3.66±1.60	p = 0.045
Systemic symptoms	5.11±1.32	p = 0.580
Activity	4.77±1.62	p = 0.066
Emotional function	5.11±1.25	p = 0.167
Worry	4.85±1.53	p = 0.221
R-S-P		
Total	58.57±19.13	p = 0.292
Physical feeling	12.77±5.14	p = 0.235
Tenderness	9.42±5.14	p = 0.061
Desire	16.1±7.26	p = 0.611
Love	8.1±3.36	p = 0.068
Communication	12.65±5.36	p = 0.471
HADS-D		
Anxiety	6.2±3.77	P = 0.204
Depression	6.24±4.36	P = 0.321

P1284
ESTABLISHMENT OF AN INTERDISCIPLINARY ONLINE EXPERT-FORUM (INXFO) SPECIALIZED IN HIV AND HEPATITIS IN GERMANY – UP-DATE 2014

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Background and Aims: The continuous progress being made in the diagnosis and treatment of HIV and hepatitis have led to an increasing complexity in the management of these infections. In response, INXFO was founded as an internet-based forum for health care providers to obtain anonymous, case-specific, free-of-charge and independent recommendations from an interdisciplinary team of experts.

Methods: INXFO went on-line in April 2012 (www.inxfo.de). After an initial, free online registration, health care providers present their case and ask their question(s) by filling in a template. The question is directed to at least one expert team-member specialized in that field, who can provide a response or obtain more information from the user. Every answer of an expert is checked by a second expert prior to release. The team of experts consists of the following specialties: cardiology, dermatology, endocrinology, gynecology, hepatology, HIV-treatment and resistance, immunology, infectious diseases, internal medicine, lipidology, microbiology, nephrology, neurology, oncology, pediatrics, pharmacy, psychiatry and virology.

Results: In 2014 (Jan-Nov), 112 questions concerning the management of complex cases were answered by INXFO, which is comparable to 2013 (n = 113). Most frequently, questions were directed at pharmacy (55.4%, an increase from 32.7% in 2013), infectious diseases (15.2%) and virology (12.5%). Drug interactions was the most common topic (35.1%), followed by co-morbidities and side effects (16.7%), therapy failure (12.3%) and hepatitis (10.5%). 71.1% of cases were reported to be HIV-infected and 5.3% and 14.9% with hepatitis B and C respectively. Questions regarding hepatitis C-infected patients as well as those directed at pharmacy nearly doubled from 2013, possibly reflecting the availability of new direct acting antivirals used in the treatment of hepatitis C.

Conclusions: Considering the complexity of HIV and hepatitis treatment, an interdisciplinary online expert-forum is a valuable tool for discussion of individual cases. A steady number of incoming questions suggests an awareness and acceptance of INXFO. The next

step will be a 'conversation-function' within the forum to allow online discussion of cases and the responses/recommendations provided by the experts.

P1285

FORECASTING THE DISEASE BURDEN OF CHRONIC HEPATITIS C IN RUSSIA

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Background and Aims: Estimated number of individuals with chronic hepatitis C (CHC) in Russia is 4,161,700; however, few are aware of their condition (1,789,500 diagnosed by 2012) and even fewer are treated (5,500 in 2010). This analysis projected future disease burden and developed four treatment scenarios to control CHC related disease burden in Russia.

Methods: Using a modelling approach, the hepatitis C virus (HCV) infected population and future disease progression was quantified. Baseline variables included: viraemic prevalence, age and gender distribution, diagnosis rate, treatment rate, disease progression and sustained virologic response (SVR) rates. Data were collected from the literature and through expert interviews.

Results: The number of prevalent HCV infections is projected to increase 36% by 2030. In addition, the number of individuals with decompensated cirrhosis, hepatocellular carcinoma (HCC) and liver related deaths (LRD) are estimated to increase 175%, 205% 220% and 215%, respectively. There are an estimated 236,000 new infections annually.

If SVR is increased, but the number of treated patient stays the same and treatment is restricted to individuals with a fibrosis stage of ≥F1 (as today), then there was only a 1% decrease in the number of HCC cases, LRD, and decompensated cirrhosis by 2030. Restricting the treatment to ≥F3 will lead a 4–5% reduction in morbidity and mortality.

Combining prevention with treatment resulted in the most substantial impact. Reducing annual incidence to 96,000 in 12 years as well as increasing treatment to 123,800 with high SVR therapies resulted in a 40% reduction in viraemic individuals. Additionally, HCC cases, liver related deaths and decompensated cirrhosis decreased by 65%, 68% and 74%, respectively.

Delaying prevention and treatment by two years resulted in a reduction in viraemic individuals, HCC cases, liver related deaths and decompensated and compensated cirrhosis by 34%, 54%, 57% and 64%, respectively. In addition, the delayed scenario resulted in an estimated 20,000 more liver related deaths.

Conclusions: A substantial reduction in CHC related disease burden is possible with increases in SVR and treatment numbers while lowering the number of new HCV infections. These results could inform the development of effective disease management in Russia.

P1286

LOW PREVALENCE OF POSITIVE VIRAL SEROLOGY FOR HBV AND HCV AMONG A GENERAL DUTCH ELDERLY POPULATION: RESULTS FROM THE ROTTERDAM STUDY

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Background and Aims: Worldwide the prevalence of chronic HBV infection is approximately 7%, whereas the prevalence of chronic HCV infection is 3%. Recently, screening for HCV among babyboomers was implemented in the United States. In the Netherlands, prevalence of HBV and HCV is much lower (0.24% for chronic HBV and 0.22% for chronic HCV infection), with a higher prevalence among first-generation migrants. Therefore we assessed the prevalence of HBsAg and anti-HCV positivity in the Rotterdam Study, a population-based cohort consisting of elderly participants of almost exclusively Caucasian ethnicity.

Methods: The Rotterdam Study is a large population-based cohort study of subjects ≥45 years at start of the study. Screening for HBV and HCV was performed with an anti-HCV ECLIA (Cobas®, Roche) and HBsAg ECLIA (Cobas®, Roche). Liver fibrosis was assessed with transient elastography (Fibroscan, Echosens®).

Results: In total, 6036 participants were included in this study. Mean age was 69.5±9.0 years and 43.1% were male. Median ALT was 19 (15–24) U/L. At abdominal ultrasonography, steatosis was present in 35.6%. Median liver stiffness (LS) (n = 4284) was 4.8 (3.9–5.9) kPa. In the total cohort, anti-HCV was positive in 34 participants (0.56%) and HBsAg positivity was observed in 12 participants (0.20%). Borderline positivity was encountered in 8 (0.13%) and 7 (0.12%) patients for anti-HCV and HBsAg respectively. Prevalence of positive viral serology in babyboomers (born 1945–1965, n = 2635) was 0.38% for HCV and 0.30% for HBV. Mean age of the 45 participants with positive viral serology in the total cohort was 71.1±8.3 years and 62.2% were male (vs 42.9% in those without positive viral serology, p = 0.009). Median ALT was 20 (16–25) U/L. Steatosis was present in 40%. Median LS (n = 31) was 5.6 (4.2–6.4) kPa vs 4.8 (3.9–5.9) kPa in those without positive viral serology (p = 0.013). Two participants (6.5%) had LS ≥9.5 kPa. Three participants (6.8%) with positive serology were of non-caucasian ethnicity compared to 4.8% in the total cohort. Ten (22.2%) participants had diabetes mellitus, this prevalence was 12% in the total cohort (p = 0.035).

Conclusions: The occurrence of positive viral serology for hepatitis B and C in a general elderly Western population, including almost exclusively participants of Caucasian ethnicity, was very low with a prevalence of 0.2% and 0.6% respectively. Therefore, screening for HBV and HCV in the general Caucasian elderly population, including babyboomers, in the Netherlands should not be recommended.

P1287

PHYLOGENETIC ANALYSIS OF AN EPIDEMIC OUTBREAK OF ACUTE HEPATITIS C IN HIV-INFECTED PATIENTS BY MASSIVE SEQUENCING

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Background and Aims: The incidence of acute hepatitis C among HIV-infected men who have sex with men (MSM) has significantly

increased in recent years. This increase may be due to factors such as high HCV viral load in blood and semen, sex with risk of mucosal damage, a higher number of sexual partners, presence of concomitant ulcerative sexually transmitted diseases and the use of recreational drugs. The aim of our study was to investigate the dynamics of HCV transmission in an outbreak of acute hepatitis C in HIV-infected MSM in Barcelona.

Methods: Between 2008 and 2013, 113 cases of acute hepatitis C in HIV-infected MSM were diagnosed in the Infectious Diseases Unit, Hospital Clínic, Barcelona. Phylogenetic analysis of the HCV NS5B gene was performed in a total of 70 patients. Viral RNA was extracted from serum samples collected from each patient at the time of diagnosis. Massive sequencing was performed using the Roche 454 GS Junior platform. To define possible transmission networks, phylogenetic trees and multidimensional scaling maps were constructed from genetic distance matrices (Da).

Results: At the time of diagnosis of acute hepatitis C, 53 of the 70 (76%) patients included in the study were receiving antiretroviral therapy. HIV viral load was undetectable in 48 patients (69%) and the mean CD4 cell count was 923 cells /ul. HCV viral load was 6.37 log IU/mL (range 3.73–6.99). Thirty-five of 53 (66%) patients treated with pegIFN and ribavirin achieved a sustained virological response. The prevalence of HCV genotypes was: 4d 51% (n = 36), 1a 40% (n = 28), 1b 7% (n = 5) and 3a 1% (n = 1). Phylogenetic analysis showed the existence of at least 13 monophyletic groups: 5 of genotype 1a, 2 of genotype 1b and 6 genotype 4d. Molecular analysis showed that the genetic distances between genotype 4d viruses (Da 5.42) were significantly lower than those of the subtypes 1a (Da 18.50, $p < 2.2 \times 10^{-16}$) and 1b (Da 15.25, $p < 1.1 \times 10^{-6}$). This result may suggest the existence of a single source of infection for genotype 4d and different sources for subtypes 1a and 1b.

Conclusions: HCV infection spreads rapidly among HIV-infected MSM through a local network in Barcelona. The implementation of public health campaigns and preventive measures, as well as treatment interventions with the new direct-acting antivirals will allow the development of strategies to reduce the HCV transmission of HCV within these high-risk groups.

P1288

BUDD CHIARI SYNDROME (BCS) IN FRANCE FROM A LARGE NATIONAL COHORT

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Background and Aims: To clarify the epidemiology of BCS in France on a large population basis.

Methods: For year 2010, 48 French liver units were asked to record prospectively and retrospectively adult in- and out-patients excluding known cirrhotic patients, hepatocellular carcinoma (HCC). Incidence and prevalence were calculated using the 2010 national census excluding 3 regions that did not provide exhaustive answers. Mortality and survival rates were calculated from incident cases. Clinical and laboratory data (including non questionable risk factors for venous thromboembolism) were collected.

Results: 270 patients were recorded. 90 were excluded (6 <18 yrs, 25 non-residents, 35 misdiagnosed, 10 missing files, 17 duplicates). There were 28 incident cases. Estimated incidence was 0.65 per million (pmi) in year 2010, and prevalence 4.02 pmi. 70% were women, mean age 39±1 years. Time from first symptoms to diagnosis was <7 days in 9%, and >1 month in 48%. At presentation, 73% of patients had ascites, 71% hepatomegaly, 69% abdominal pain, 55% oesophageal varices, 50% splenomegaly, and 28% a factor V <50%. Risk factors were myeloproliferative diseases (MPD) in 46% (polycythemia vera 61%, essential thrombocythaemia 28%, latent MPD 10% and myelofibrosis 1%), oral contraceptives in 33%, factor V Leiden in 15% and a local factor in 7.4%. Platelet counts were >450,000/mm^3 in 78% of MPD patients. MPD was known but untreated prior to BCS in 9/72 patients No risk factor was identified in 23% of patients and ≥2 risk factors in 24%. BMI was higher in the group without cause (25.8 kg/m^2 vs 23.5 kg/m^2, p = 0.018). Within the first year, refractory ascites occurred in 24% of cases, variceal bleeding in 10% of cases. There were 8 HCC in a mean delay of 11.2±4.2 years. Half interventional treatments were performed within the first year (15% TIPS, 13% angioplasty and 5% liver transplantation). Angioplasty, TIPS/LT one-year free survival were respectively 81.2±7.6% and 79.6±8.1%. 50% of deaths occurred the first month. In October 2014 mean survival rate was 3.5±0.4 years and global SR 69.2±9.1%.

Conclusions: In France, incidence and prevalence of BCS were at least 0.65 pmi and 4.02 pmi, respectively, in 2010, close to previous European reports. Overall mortality was higher than expected based on the data from expert referral centers. In half the patients, diagnosis is delayed beyond 1 month. Reducing this delay might be a target for improving the outcome.

P1289

UNDERSTANDING AND PREVENTING THE HCV EPIDEMIC AMONG MEN WHO HAVE SEX WITH MEN IN THE UK: A MATHEMATICAL MODELLING ANALYSIS

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Background and Aims: There is an emerging hepatitis C virus (HCV) epidemic among HIV-positive men who have sex with men (MSM). Recent UK reports indicate declines in primary and reinfection HCV incidence, despite an expanding epidemic. We use a mathematical model to understand potential drivers of recent incidence declines and predict the impact of HCV prevention interventions, including HCV interferon-free direct acting antivirals (DAAs).

Methods: A dynamic deterministic HCV transmission and treatment model among HIV-positive MSM was developed and calibrated to UK data on HCV Ab+ seroprevalence (2004–2011, UK Collaborative HIV Cohort [UK CHIC]), primary incidence (2002–2012, Public Health England), and rates of reinfection (2005 and 2011, Chelsea and Westminster Hospital cohort). Based on data, we model HCV treatment rates of 39%/22% treated within 1 year of an acute/chronic diagnosis, respectively, and 1–6%/yr thereafter (UK CHIC). We assume historic interferon-based SVR rates of 80%/40% in the acute/chronic stage, respectively, and 90% with DAAs from 2015. The model was used to understand observed declines in incidence and project the epidemic with: (a) current levels of risk and treatment (status quo), (b) double treatment rates, (c) double treatment and halved risk from 2015 (combination prevention), and (d) no treatment or historic risk reduction (counterfactual).

Results: Recent declines in primary incidence and reinfection rates suggest a 70% reduction in transmission risk from 2008–2012, with model projections indicating this will result in HCV Ab+ seroprevalence plateauing at 9% and a slight fall in chronic RNA+ prevalence to 7% among HIV+ MSM by 2025 (Figure 1a,b). Without HCV treatment or risk reductions, seroprevalence could have been 12% in 2014, rising to 14% by 2025. Doubling HCV treatment could reduce chronic prevalence from 8% to 6% over a decade, with incidence and chronic prevalence a relative 12% less than the status quo in 2025 (Figure 1b,c). Combination prevention could reduce chronic prevalence by over a third (to 5%, a quarter lower than the status quo in 2025) and reduce primary and reinfection incidence by 55–60% by 2025 (Figure 1a–d).

Conclusions: Past treatment and risk reductions in the last 6 years may have averted an expanding HCV epidemic among HIV+ MSM in the UK. Combination prevention including HCV treatment and behavioural interventions could halve incidence within a decade. Further research should identify high risk individuals to focus intervention strategies for maximum impact.

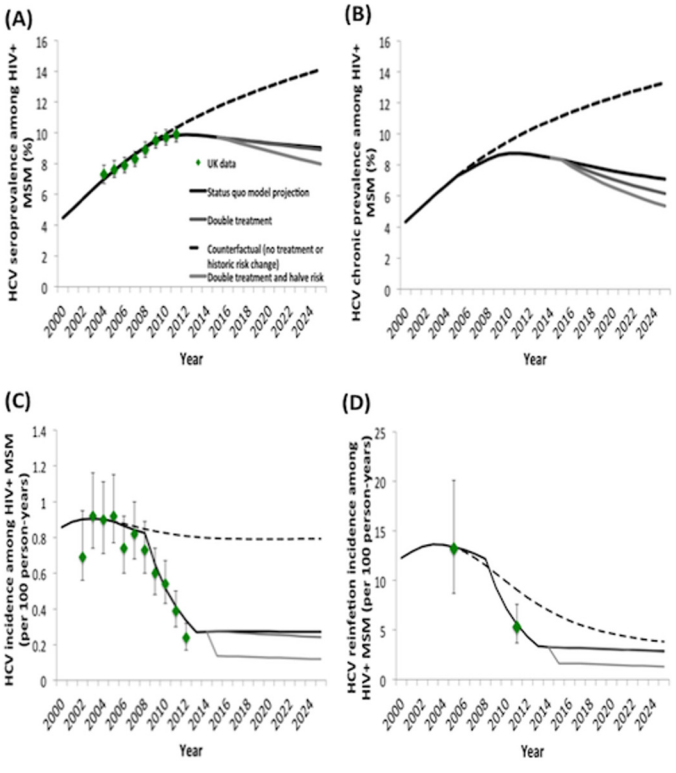

Figure 1. Model projections of the HCV epidemic among HIV-positive MSM in the UK. (A) HCV seroprevalence (Ab+); (B) HCV chronic (RNA+) prevalence; (C) HCV incidence and (D) HCV reinfection incidence. Black lines show status quo projections (continued treatment and historic risk reductions with no change since 2012), dark grey lines show double treatment from 2015, light grey lines show double treatment and halved risk from 2015, and black dashes show counterfactual of no treatment or historic risk reductions.

P1291

INCIDENCE OF BUDD CHIARI SYNDROME (BCS): AN EXHAUSTIVE NATIONWIDE FRENCH STUDY

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Background and Aims: To evaluate exhaustive incidence of BCS in France.

Methods: BCS were screened from 2007 to 2012 in French healthcare databases using their unique identifier number and the ICD-10-CM Version 2010 codes. Incident-cases were defined by the entry point as a first admission in 2012, and excluded when admission pattern was not related to BCS, or Arnold Chiari Syndrome (ACS) suspected. Mortality and incidence were calculated using the 2012 national census. Demographic data, primary and secondary risk factors, patient's location, the type and annual BCS-caseload of the centers where patients were first managed, were retrieved. Annual BCS-caseload of centers were stratified by: <5 patients/year, 5–15 patients/year, >15 patients/year.

Results: For year 2012, 258 BCS incident-cases were identified. 25 (9 ACS) were excluded. Global and primary BCS incidences were respectively 3.5 and 0.75 per million inhabitants (pmi). Patients were first managed in public sector 53.7%, university public sector 27.9%, private center 13.7% and charitable hospital 4.7%. Primary BCS were mostly first admitted in university public sector (56%). After adjusting for potential confounders, the mortality (9.8%) was only significantly influenced by age (adjusted OR = 1.031 [1.006–1.056], p = 0.008). Primary BCS mortality was 6%. Mean age was 56.1 yrs (±21) (10.3% <18) and 55.5% were male. No risk factor (RF) was identified in 32.2% and ≥2 risk factors in 13.3%. RF were inflammatory diseases 12%, alcoholic and viral cirrhosis excluding HCC 9.4%, secondary hepatic tumor (SHT) 9%, hepatocellular carcinoma (HCC) 8.1%, liver graft complications 6.4%, primary hepatic tumor excluding HCC 4.3%, mechanic or traumatic factors involving inferior vena cava 3%. Other RF were hypercoagulable state such as cancer excluding SHT 12%, myeloproliferative

diseases (MPD) 8.6%, other coagulation disorders 4.7%, pregnancy complications 3% and rare diseases 2.6%. Among the 50 primary BCS, mean age was 43.8 yrs (±20) (3 <18) and 64% were female. RF were inflammatory diseases 36%, MPD 28%, other coagulation disorders 16%, pregnancy complications 14%, rare diseases 12% and mechanic or traumatic factors involving inferior vena cava 6%.
Conclusions: In France in 2012, exhaustive incidence of BCS was 3.5 pmi. This study confirms that incidence of primary BCS is low, close to expert centers' evaluation in 2010.

P1292
INCIDENCE OF HEPATITIS C IN AN EUROPEAN LOW THRESHOLD METHADONE PROGRAM

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Background and Aims: Data on incidence of hepatitis C virus (HCV) infection in Europe are sparse. Where documented, injecting drug use is a major transmission route for HCV infections.
We aimed to estimate the incidence of HCV infection in an ambulatory medical and psychosocial program with low threshold methadone administration in Lisbon, Portugal (LTMP-Lx).
Methods: At LTMP-Lx, serological status for HCV and human immunodeficiency virus (HIV) are collected prospectively from every individual in the cohort. Negative serologies are repeated annually during follow-up. Drug use and other risk behaviors are accessed during periodical consultations within the program. We analyzed serologies from individuals entering the program between 2001 and 2013, and estimated the incidence of HCV seroconversion in the whole cohort and its subgroups. Statistical analysis was performed with Microsoft Office® Excel 2007 and STATA® 12.1.
Results: During the 12 years of observation, 5038 individuals in the program were tested for HCV antibodies. A total of 1032 individuals – 801 (77.6%) males, median age at program entry 35 [18; 63] years, 173 (16.8%) have ever injected drugs – had a baseline negative HCV serology and at least one further serology. Of those, 85 (8.2%) had a positive HIV baseline serology.
Ninety-one individuals (8.8%) seroconverted for HCV, during 3852.5 person-years (PY) [HCV seroconversion incidence 2.36/100PY (95% CI 1.92–2.90/100PY)].
Seroconversion incidence was 2.22/100PY (95% CI 1.75–2.81/100PY) among males, 2.93/100PY (1.95–4.41/100PY) among females; 7.59/100PY (95% CI 5.13–11.23/100PY) among HIV positive and 1.88/100PY (95% CI 1.47–2.39/100PY) among HIV negative individuals. Incidence was 0.78/100PY (95% CI 0.53–1.15/100PY) among individuals with non-injecting drug use in their lifetime and 12.15/100PY (95% CI 9.53–15.49/100PY) among individuals who ever injected drugs.
Conclusions: At this european low threshold methadone administration program, HCV seroconversion incidence is low in non-injecting drug users and is significantly higher among injecting drug users and HIV positive individuals.

P1293
RESULTS OF A GLOBAL SURVEY OF HEPATITIS C PATIENTS: THE IMPACT OF INFECTION, THE EXPERIENCE OF THE PATIENT PATHWAY AND THE BARRIERS TO, AND SIGNIFICANCE OF, A CURE

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Background and Aims: Somewhere between 80 and 150 million people are living with hepatitis C so a survey was conducted to establish the impact on their lives, socially, economically, physically and psychologically, the impact of being cured of the virus and the experience of the patient pathway.
Methods: A questionnaire was developed with input from both patients and physicians and distributed during 2014 to patients online and in physical form through patient organisations and physician associations. It was available in 35 languages. Most questions were multiple choice, but those addressing the barriers to, and impact of, a cure were free text.
Results: Half or more of respondents said that living with hepatitis C had a significant or very significant negative impact on their emotional life (romantic or family relationships), on their physical health and particularly on their psychological well-being. Around one third felt that it had negatively impacted their education or career prospects and a similar proportion had not told even their close family about their status. Half felt that nurses did not understand this impact and a third that doctors did not. Of those that knew about hepatitis before their diagnosis a tiny minority had heard about it through government awareness campaigns and only a third were tested at their own or their doctor's initiative. Of those who told their doctor about hepatitis C symptoms, two-thirds were not offered a test. Half of respondents were unsatisfied with the information given to them on diagnosis. Almost all respondents had tried herbal remedies to help with their hepatitis C, most either to slow disease progression or to ameliorate the symptoms. The key barrier for people in addressing their hepatitis C was treatment, either access to it or the side effects of interferon-based therapy. The predominant description of the impact of achieving a cure was 'life'.
Conclusions: Living with hepatitis C has a major impact on all aspects of a person's life but patients do not perceive that healthcare workers, especially nurses appreciate this. The provision of information prior to diagnosis, especially from governments, as well as on diagnosis clearly needs to be improved. Equally, treatment needs to move rapidly to interferon-free regimens and access assured for all, as this will remove the two key barriers for patients. This is particularly important because for patients a cure is not seen as a 'nice-to-have'; it means 'life'.

Clinical trials in progress

P1309
A RANDOMIZED, OPEN-LABEL, MULTICENTER, CONTROLLED STUDY TO ASSESS SAFETY AND EFFICACY OF ELAD® HUMAN CELL-BASED BIO-ARTIFICIAL LIVER SUPPORT SYSTEM (ELAD®) IN SUBJECTS WITH ALCOHOL-INDUCED LIVER DECOMPENSATION (AILD)

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Background and Aims: Treatment options for patients with AILD are limited, leading to significant morbidity and mortality. ELAD® is designed to provide liver support continuously for up to 5 days to a subject with compromised liver function and allow time for the native liver to regenerate by circulating patient plasma through a hollow fiber cartridge containing metabolically active, immortalized VTL C3A human liver cells. Based on preliminary findings from a subset of subjects with AILD enrolled in a prior Phase 2 study (VTI-206), the aim is to provide data on the safety and clinical utility of ELAD in a larger, prospectively defined population with AILD. The

primary endpoint will evaluate overall survival of subjects with a clinical diagnosis of AILD up to at least Study Day 91. The secondary objectives are to determine the proportion of survivors at Study Days 28 and 91. Exploratory objectives are to evaluate the ability of ELAD to stabilize liver function, measured using the Model of End-stage Liver Disease (MELD)-based time to progression (TTP) up to Study Day 91, and the proportion of progression-free survivors (PFS) up to Study Days 28 and 91.

Methods: Eligibility requirements were established in order to replicate the AILD population in VTI-206 as follows:

Key inclusion criteria:

1. Age ≥18 years;
2. Total bilirubin ≥8 mg/dL;
3. A clinical diagnosis of AILD, based upon evidence (by lab test, medical history, or family interview) of a clinical judgment of a temporal (6 weeks or less) and causal relationship between use of alcohol and this onset of symptoms;
4. Subjects meeting inclusion criteria 1 through 3 will be classified as having either:
   a. Severe acute alcoholic hepatitis (AAH), with:
      i. Medical history of alcohol abuse; AND
      ii. Maddrey score of ≥32; AND
      iii. AAH documented by either:
         1. Confirmatory liver biopsy, OR
         2. Two or more of the following:
            a. Hepatomegaly,
            b. AST > ALT,
            c. Ascites,
            d. Leukocytosis (WBC count above lab normal at site), OR
   b. Alcohol-induced decompensation of chronic liver disease that is not acute alcoholic hepatitis as defined above), with:
      i. MELD score of 18–35; AND
      ii. Underlying chronic liver disease documented by:
         1. Liver biopsy, AND/OR
         2. Laboratory findings, AND/OR
         3. Medical history;
5. Not eligible for liver transplant during this hospitalization.

Key exclusion criteria:

1. Platelets <40,000/mm^3, INR >3.5;
2. Chronic renal failure;
3. MELD score >35;
4. Septic shock, major hemorrhage, spontaneous bacterial peritonitis with uncontrolled systemic infection, hemodynamic instability;
5. Significant concomitant disease;
6. Previous liver transplant;
7. Have a Do Not Resuscitate or a Do Not Intubate (DNR/DNI).

Target age based on the VTI-206 population is 45–55 and baseline target MELD is 27–29 for the current study. A total of 200 evaluable subjects meeting these requirements will be randomly assigned in a 1:1 ratio to receive either standard of care treatment for AILD plus treatment with the ELAD System (ELAD group) or standard of care of treatment for AILD alone (Control group). ELAD treatment will take place for a maximum of five 24-hour periods unless the discontinuation criteria are met. It was anticipated that enrollment would take approximately 2 years.

Results: The first subject was enrolled in March 2013 and as of November 12, 2014, 175 subjects have been enrolled in 51 clinical sites in the United States, Europe and Australia. Based on data captured as of this date in the Electronic Data Capture system for the study, major demographics/baseline variables include: Age (45.16±10.10, n = 174), MELD (27.23±3.94, n = 172), Maddrey (73.15±25.39, n = 173), bilirubin (24.79±9.39 mg/dL, n = 173), INR (2.04±0.52, n = 172), creatinine (0.99±0.74 mg/dL, n = 172), PT (22.42±5.30 seconds, n = 173).

Conclusions: Trial enrollment has proceeded in accord with anticipated timelines. Average age and MELD at baseline are within the trial target ranges established during VTI-206.

P1310

ADJUVANT CHEMOTHERAPY WITH GEMCITABINE AND CISPLATIN COMPARED TO OBSERVATION AFTER CURATIVE INTENT RESECTION OF CHOLANGIOCARCINOMA AND MUSCLE INVASIVE GALLBLADDER CARCINOMA (ACTICCA-1) – A RANDOMIZED, MULTIDISCIPLINARY, MULTINATIONAL PHASE III TRIAL

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Background and Aims: Despite complete resection, disease free survival (DFS) of patients with cholangiocarcinoma (CCA) is less than 65% after 1 year and not more than 35% after 3 years. For muscle invasive gallbladder carcinoma (GBCA) prognosis is even worse, with an overall survival (OS) of only 30% after 3 years. Thus, evaluation of adjuvant chemotherapy (CTx) in biliary tract cancer (BTC) in a large randomized trial is urgently warranted.

Methods: ACTICCA-1 is a randomized, multidisciplinary, multinational phase III investigator initiated trial (EudraCT number 2012–005078–70). With respect to data obtained in the ABC-02 trial, we selected the combination of cisplatin and gemcitabine for 24 weeks as investigational treatment. Based on adjuvant trials in pancreatic cancer (e.g. ESPAC IV) with a comparable postoperative recovery time, inclusion of patients within a maximum interval of 16 weeks between surgery and start of CTx was chosen. Due to the different prognosis and treatment susceptibility of muscle invasive carcinoma, two separate cohorts for CCA and GBCA were included to capture the expected treatment effects. Randomization will be stratified for lymph node status for both cohorts and localization for CCA. The primary endpoint is DFS and secondary endpoints include OS, safety and tolerability of adjuvant CTx, quality of life, and patterns of disease recurrence. For CCA, DFS 24 months post surgery (DFSR@24) is expected to be 40% without CTx. Adjuvant gemcitabine and cisplatin should increase DFS@24 to 55% to be regarded as clinically relevant. With a power of 80% and a significance level of 5%, 271 evaluable study patients have to be followed for 24–28 months to observe 166 events. For GBCA, adjuvant CTx should increase DFSR@24 from 35% to 55% to be relevant; thus, 154 evaluable study patients have to be monitored for 24–28 months to observe 90 events. In both cohorts, randomization will be 1:1 with adjuvant CTx for 24 weeks and imaging every 12 weeks. In 2014, the study has been started in Germany (funded by the Deutsche Krebshilfe and supported by medac GmbH). Site activation in Germany will continue in 2015. Furthermore, the Netherlands (funded by the Dutch Cancer Society), Australia, Denmark and the United Kingdom (funded by Cancer Research UK) will join in 2015.

Conclusions: The randomized, multinational, multidisciplinary phase III ACTCCA-1 trial will establish the role of adjuvant CTx

with gemcitabine and cisplatin in comparison to observation in patients with BTC.

P1311

THRIL; PILOT STUDY EVALUATING THE SAFETY AND EFFICACY PROFILE OF REGULATORY T CELL THERAPY IN LIVER TRANSPLANT RECIPIENTS

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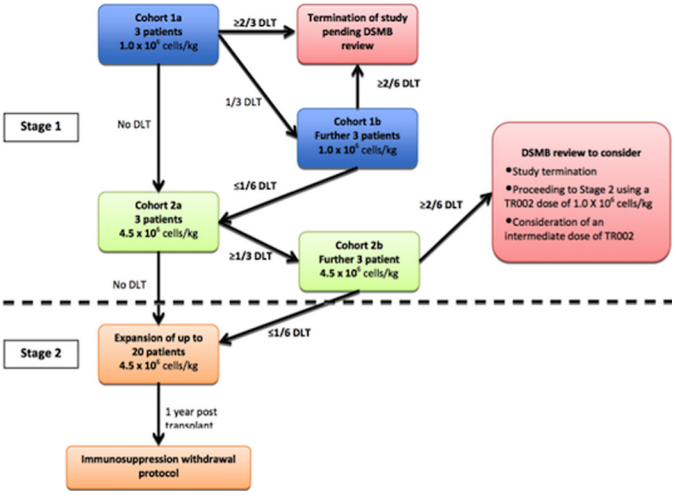
Background and Aims: Protracted immunosuppression following liver transplantation is accompanied by significant morbidity and mortality and negatively impacts on the long term survival of liver transplant recipients. A proportion of liver transplant recipients develop a state of 'operational' tolerance and can discontinue all immunosuppressive treatment without undergoing rejection. However, this phenomenon is exceedingly rare over the first years after transplantation. $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs) are ideal candidates for therapeutic strategies aimed at inducing tolerance in organ transplantation, but the evidence for the use and efficacy of Treg therapy in this setting is absent.
ThRIL is the first combined Phase I/IIa clinical trial of Treg immunotherapy worldwide in the setting of liver transplantation. Stage 1 will evaluate the safety and dose limiting toxicities of TR002 administration to adult de novo primary liver transplant recipients. Stage 2 will evaluate the efficacy of TR002 administration in allowing the complete discontinuation of immunosuppressive therapy.
Methods: A single site, open-label, uncontrolled, phase I/IIa clinical trial in subjects undergoing liver transplantation, in which liver transplant recipients will receive a single infusion of an in vitro expanded autologous regulatory T cell therapy (TR002). Participants will be adults on the King's College Hospital liver transplant waiting list with chronic liver disease and no history of autoimmunity or active HCV infection. Recipient blood will be collected immediately before transplantation for TR002 manufacture. Participants will receive immunosuppression with rATG in addition to Prednisolone and Tacrolimus. Conversion to Sirolimus based immunosuppression will occur at 8 weeks post-transplantation.



In Stage 1, two cohorts of three evaluable patients will be recruited, with cohort 1 receiving a low dose TR002 infusion (1.0×10^6 cells/kg) and cohort two receiving a higher dose TR002 infusion (4.5×10^6 cells/kg) at week 13. The number of dose limiting toxicities up to one month after cell infusion will determine the requirement to expand cohort 1 and/or 2 to six patients as shown in the figure. Protocol liver biopsies will be performed at week 13 and 52. In stage 2, a maximum of 20 patients who have received the highest tolerated dose of TR002 will initiate gradual immunosuppression discontinuation at 12 months. Drugs will be withdrawn over a 6 month period, and patients will be followed until month 24.

P1312

ALFAPUMP SYSTEM VERSUS TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT AND PARACENTESIS IN THE TREATMENT OF ASCITES. A MULTICENTRE RANDOMISED CONTROLLED STUDY (AGUA-TRIAL)

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Background and Aims: Ascites represents a complication of liver cirrhosis that becomes refractory to medical treatment in 5–10% of patients. The treatment of choice is repeated paracentesis or the implantation of a transjugular intrahepatic portosystemic shunt (TIPS). However, complications such as bleedings, hepatic encephalopathy and liver failure limit applicability of TIPS. The alfapump® system from Sequana Medical is a device implanted at subcutaneous level, which is able to move the ascitic fluid from the peritoneal cavity to the urinary bladder. Usually the device is implanted in patients with advanced stages of liver cirrhosis if TIPS is contraindicated. However, complications like renal insufficiency and infections largely result from this late disease stage.
Aim: The purpose of the Agua-Trial is to evaluate the efficacy of the alfapump in patients with cirrhosis presenting with recurrent or refractory ascites. Two patient populations are studied with different aims:

1. patients with indication to TIPS – the aim is to show that alfapump is non-inferior to TIPS in reducing of the need for paracenteses.
2. patients with contraindication to TIPS – the aim is to show that alfapump is superior to repeated paracenteses, reducing the need for paracenteses.

Methods: Agua-Trial is a prospective, randomized, open multicenter international trial consisting of two sub-studies with 200 patients in part 1 and 60 patients in part 2. The main inclusion criteria are: age >18 years, liver cirrhosis, refractory or recurrent ascites, written informed consent. For sample size calculation an average number of paracenteses per quarter of 0.817 for alfapump and 1.17 for TIPS and a power of 90% was assumed.
For both sub-studies, the primary endpoint is the average number of paracenteses per quarter (three months) documented over 4 quarters (12 months). Secondary objectives include treatment related complications, transplant-free survival, need for albumin

substitution and quality of life. Patients will be followed-up for 24 months, with the primary endpoint being assessed after 12 months. The trial will start in 2015.
Conclusions: Agua-Trial is the first large-sale clinical trial to evaluate the efficacy as well as the complications of alfapump in comparison with TIPS in patients with recurrent or refractory ascites qualifying for both treatments. Agua-Trial will provide important information about the clinical course of this condition and may have significant impact on treatment strategies in these patients

P1313
WTX101–201: PHASE 2 STUDY OF BIS-CHOLINE TETRATHIOMOLYBDATE IN NEWLY DIAGNOSED WILSON DISEASE PATIENTS

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Background and Aims: Wilson Disease (WD) is a rare autosomal-recessive disorder of impaired hepatic copper excretion caused by mutations in the ATP7B gene, resulting in copper accumulation in various organs, including the liver and the brain. WTX101 (bis-choline tetrathiomolybdate) is a novel de-coppering agent with a unique mechanism of action (MoA) in development for treatment of WD. WTX101 targets improved control of Cu due to the rapid formation of irreversible Cu-tetrathiomolybdate-protein complexes leading to a rapid de-coppering without mobilizing free Cu that could cause tissue toxicity, including neurological deterioration. Tetrathiomolybdate has been studied in several clinical trials in WD patients using ammonium tetrathiomolybdate. The study will evaluate if individualised BID oral dosing of WTX101 with an up-titration design in the initial de-coppering phase is safe and efficacious in lowering free copper levels (NCC, adjusted for plasma Mo concentration) as well as maintaining these lowered free copper levels using QD dosing. It will also provide data on additional free Cu endpoints, 24-hour urinary copper and neurological outcome with the UWDRS.
Methods: This is a Phase 2, open-label, non-controlled, 24 week study conducted in the EU and US. An individualised WTX101 dosing regimen is administered with PPI in newly diagnosed symptomatic (neurological, stable hepatic or combined) WD patients aged 18 and older with elevated NCC levels who have been treated for ≤28 days with chelation therapy or zinc therapy. Eligible subjects will receive WTX101 as out-patients at doses ranging from 30–300 mg per day along with omeprazole 20 mg per day. The primary endpoint is the proportion of patients who achieve normalised levels of NCC (≤2.3 μM) or reach a reduction of at least 25% in NCC. Secondary endpoints include safety and tolerability, change in and time to normalisation of NCC levels, copper endpoints (exchangeable copper, profiling of Mo/Cu/Protein tripartite complex species, and 24-hour urinary copper), hepatic status with ALT, AST, INR and bilirubin measurement, neurological assessment (UWDRS), psychiatric assessment (MINI Tracking), clinical symptom status (CGI) and QOL/PRO endpoints (EQ5D, MMAS-8, TSQM). PK of WTX101 will be measured by plasma total and free elemental Mo.
Conclusions: The unique mode of action suggests that WTX101 may be an effective treatment option for WD patients having a lower potential for neurologic deterioration while once daily dosing offers improve compliance.

P1314
GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) TO TREAT ACUTE-ON-CHRONIC LIVER FAILURE: A MULTICENTER RANDOMIZED TRIAL (GRAFT STUDY)

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Background and Aims: ACLF is a condition with dismal prognosis, for which apart from OLT no therapeutic options exist. A treatment approach that helps to recover from this acute intercurrent event and hereby improves the very poor short-term survival would be of major clinical importance. Recently two small randomised trials have shown the potential of G-CSF in this patient population markedly improving patients' outcome. However, these results need to be confirmed in a large multicenter trial.
Aim: The purpose of the GRAFT Study is to evaluate the efficacy of G-CSF in patients with ACLF.
Methods: GRAFT Study is a prospective and open multicenter trial randomizing 292 patients with ACLF in two arms. All patients will receive standard of care according to current guidelines. Patients in the experimental arm receive in addition twelve doses of G-CSF (5 μg/kg daily) administered subcutaneously on day 1–5, then every 3rd day until day 26. Inclusion criteria are: age >18, ACLF according to the diagnostic criteria of the CANONIC Study and written informed consent. We aim to detect an absolute difference of 20% in transplant-survival at day 90. With these assumptions, a total of 262 patients have to be analysed to achieve a power of 90% at a significance level of 5% using a two-sided log-rank test. Due to the short observation period for the primary endpoint, we expect a drop-out rate not exceeding 10%. Taking this into account, 292 patients will be randomized. The primary endpoint is the transplant-free survival up to 90 days. Secondary objectives include the overall survival, complications of ACLF, rate of infections, liver function and the duration of hospital stay. Patients will be followed-up for 12 months. The trial will start in 2015.
Conclusions: GRAFT Study is the first large-scale clinical trial evaluating the efficacy as well as the safety of G-CSF in patients with ACLF. GRAFT Study will provide important information about the clinical course of this condition and may have significant impact on treatment strategies in these patients.

P1315
DESIGN OF THE INCA TRIAL (IMPACT OF NOD2 GENOTYPE-GUIDED ANTIBIOTIC PREVENTION ON SURVIVAL IN PATIENTS WITH LIVER CIRRHOSIS AND ASCITES): PROSPECTIVE, DOUBLE-BLIND, RANDOMISED CLINICAL TRIAL EVALUATING THE IMPACT OF GENOTYPE-STRATIFIED CARE

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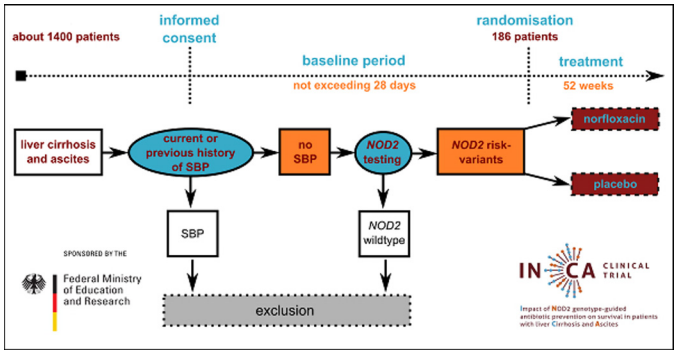
Background and Aims: Patients with liver cirrhosis suffer from a highly elevated risk to develop bacterial infections that significantly decrease survival rates. One of the most relevant infections is spontaneous bacterial peritonitis (SBP). Recently, we reported that *NOD2* germline variants are associated with the development of infectious

complications and mortality in patients with cirrhosis (Appenrodt et al., 2010; Lutz et al., 2014). The *aim* of the INCA trial is to investigate whether survival of this genetically defined high-risk group of patients with cirrhosis characterised by the presence of *NOD2* variants is improved by primary antibiotic prophylaxis of SBP.

Methods: The INCA trial is a double-blind and placebo-controlled clinical trial with two parallel treatment arms (arm 1: norfloxacin 400 mg once daily; arm 2: placebo once daily; 12 months treatment and observational period). Balanced randomisation of 186 eligible patients with stratification for the protein content of the ascites (<15 vs. ≥15 g/l) and the study site is planned. As a multicentre national study patients are recruited at at least 13 centres throughout Germany. Key inclusion criterion is the presence of a *NOD2* risk variant in patients with liver cirrhosis who developed ascites, the most important exclusion criteria are a current SBP or previous history of SBP and any long-term antibiotic prophylaxis (Figure 1). The primary endpoint is overall survival after 12 months of treatment. Secondary objectives are to evaluate whether the frequencies of SBP and other clinically relevant infections necessitating antibiotic treatment as well as the total duration of unplanned hospitalisation due to cirrhosis differ in both study arms. Recruitment has started in March 2014. The study is registered in the German Clinical Trials Register (DRKS00005616).

Conclusions: Preventive strategies are required to avoid life-threatening infections in patients with liver cirrhosis, but unselected use of antibiotics can trigger resistant bacteria and worsen outcome. Thus, individualised approaches that direct intervention only to patients with the highest risk are urgently needed. This trial meets this need by suggesting stratified prevention based on genetic risk assessment. The INCA trial is among the first studies aiming to rapidly transfer and validate information on individual genetic risk into clinical decision algorithms for patients with end-stage liver disease.



P1316

EFFECTS OF LONG-TERM ADMINISTRATION OF MIDODRINE AND ALBUMIN IN THE PREVENTION OF COMPLICATIONS IN PATIENTS WITH CIRRHOSIS ON WAITING LIST FOR LIVER TRANSPLANTATION. A RANDOMIZED, MULTICENTER, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL. MACHT STUDY

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Background and Aims: Patients with cirrhosis awaiting liver transplantation frequently develop complications of the disease that increase mortality in the waiting list. Many of these complications are related to an impairment of circulatory function that causes effective hypovolemia. The hypothesis of the study is that the administration of midodrine, an oral vasoconstrictor, associated with albumin, may improve circulatory function and prevent complications of cirrhosis while awaiting for transplantation.

Endpoints: The primary endpoint of the study is to evaluate the incidence of complications (kidney failure, hepatic encephalopathy, hyponatremia, bacterial infections and gastrointestinal bleeding) in patients with cirrhosis and ascites awaiting liver transplantation. Secondary end points are: control of ascites, relationship between development of complications and activity of vasoconstrictor systems (renin, aldosterone, norepinephrine) and cytokines (TNF, IL6, IL10), quality of life, presence and outcome of minimal hepatic encephalopathy (MHE), and survival.

Study design: Randomized, multicenter, double-blind, placebo-controlled trial that will include 194 patients with cirrhosis and ascites awaiting liver transplantation. Inclusion criteria are patients with cirrhosis and ascites just enrolled in the waiting list for liver transplantation. Exclusion criteria include patients with HIV infection, TIPS and patients with contraindications for midodrine (i.e., arterial hypertension, heart disease). Included patients are randomized to receive treatment with midodrine (15–30 mg/day) associated with intravenous albumin (40 g/15days) (treatment group) or placebo (saline and placebo tablets; placebo group). Randomization will be 1:1 between groups. Randomization will be stratified according to the inclusion center and to the presence of kidney failure at inclusion (defined as serum creatinine >1.2 mg/dL). Treatment will be administered during one year or until liver transplantation. Liver and kidney function tests, vasoconstrictor systems and cytokines will be determined at baseline and at months 1, 3, 6, 12. At study inclusion neuropsychological test (PHES score) will be performed to diagnose MHE. This test will be repeated at months 3, 6, 12. Finally, quality of life questionnaires will be performed at baseline and at months 3, 6, 12. During follow-up the number of paracenteses required and the development of complications of cirrhosis will be recorded

P1317

EFFICACY OF URSODEOXYCHOLIC ACID AS A VOLUME REDUCING TREATMENT FOR SYMPTOMATIC POLYCYSTIC LIVER DISEASE: AN INTERNATIONAL, MULTICENTER, RANDOMIZED CONTROLLED TRIAL

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Background and Aims: A large proportion of patients with polycystic liver disease (PLD) due to autosomal dominant polycystic kidney disease (ADPKD) or autosomal dominant polycystic liver disease (ADPLD) suffer from symptomatic hepatomegaly. Conventional invasive therapies may cause serious complications and have high recurrence rates. Medical treatment with somatostatin analogues that aim to diminish increased intracellular cAMP levels in polycystic cholangiocytes, show modest beneficial effects and considerable side effects. The bile acid ursodeoxycholic acid (UDCA) is FDA-approved for the treatment of the most common cholestatic liver disease, primary biliary cirrhosis. Experimental evidence suggests that UDCA reduces cystogenesis in a rodent model of PLD by restoring diminished intracellular free calcium

levels in polycystic cholangiocytes. We hypothesized that UDCA may be effective in reducing total liver volume (TLV) in PLD patients.

Methods: This international, multicenter, randomized, controlled clinical trial evaluates the effect of 24 weeks of UDCA as a volume reducing treatment. Eligible patients are symptomatic PLD patients that have a TLV ≥2500 mL. Primary outcome is change in TLV determined by CT volumetry. Secondary outcomes are change in symptoms and health-related quality of life. Moreover, safety and tolerability of the drug will be assessed. Patients will be randomized through a 1:1 allocation. The intervention group receives UDCA 15–20 mg/kg/day for 24 weeks, the control group will receive standard care. 34 patients will be included for a power of 80% (alpha 0.05) to detect a change in difference in TLV of at least 4% in favor of the UDCA-treated group.

Conclusions: Treatment of PLD with UDCA appears promising based on experimental observations. A 4% difference in TLV in favor of UDCA treatment versus standard care would indicate comparable efficacy of UDCA to that of somatostatin analogues at lower costs, less side effects and contra-indications and a more acceptable route of admission. In case of a positive result, further research should focus on the molecular mechanism of action of UDCA in PLD, dose-response relationship and combination therapy of somatostatin analogues with UDCA to evaluate synergistic effects.

P1318

PHASE I DOSE ESCALATION STUDY OF THE SAFETY, IMMUNOREGULATORY ACTIVITY, PHARMACOKINETICS, AND PRELIMINARY ANTITUMOR ACTIVITY OF NIVOLUMAB IN ADVANCED HEPATOCELLULAR CARCINOMA IN PATIENTS WITH OR WITHOUT CHRONIC VIRAL HEPATITIS

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Background and Aims: Sorafenib is the standard of care and sole option for patients with advanced hepatocellular carcinoma (HCC) who are not candidates for curative resection or other hepatic regional therapies. The immune checkpoint inhibitor, programmed death-1 (PD-1), reduces immune response to tumor cells when the PD-1 receptor interacts with its ligands, PD-L1 and PD-L2, to down-regulate T-cell activation [1]. HCC overexpressing PD-L1 is aggressive with a poor prognosis [2]. Nivolumab, a fully human IgG4 monoclonal antibody, blocks interaction between PD-1 and its ligands and has demonstrated antitumor activity in solid tumors [3] and hematologic malignancies [4]. This study (CA209-040/NCT01658878) will evaluate the safety and antitumor activity of nivolumab in patients with advanced HCC with or without chronic viral hepatitis.

Methods: This open-label, 3-arm, dose escalation study will enroll patients with advanced histologically confirmed HCC, ECOG PS of 0 or 1 and Child–Pugh Class B7 or less who are not candidates for surgery or locoregional therapies and had progressive disease on or were intolerant of at least 1 prior therapy or refused sorafenib. Patients with encephalopathy, prior or current clinically significant ascites, recent variceal bleeding, or co-infection with either hepatitis B and C or hepatitis B and D are ineligible. Study arm assignment is dependent on HCC risk factors: no active hepatitis virus infection (non-viral HCC); chronic hepatitis C (HCC-HCV); chronic hepatitis B (HCC-HBV). Nivolumab dose levels for non-viral HCC and HCC-HCV patients are 0.3, 1, 3 and 10 mg/kg. HCC-HBV patients' dose escalation levels are 0.1, 0.3, 1, 3, and 10 mg/kg. Dose escalation will proceed independently in each arm (3+3 design). Nivolumab will be administered as a 1 hour infusion on days 1, 15, and 29 of each 6-week cycle for up to 2 years. In the dose expansion phase, 200 additional patients will receive nivolumab: 100 non-viral HCC and 50 HCC-HCV patients at the 3 mg/kg dose and 50 HCC-HBV patients at 3 mg/kg or the maximum tolerated dose. Primary endpoints are incidence of worst adverse events and incidence of clinical laboratory test abnormalities. Secondary endpoints include assessments of nivolumab antitumor activity, pharmacokinetics and immunogenicity.

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P1319

ALFAPUMP® SYSTEM VERSUS LARGE VOLUME PARACENTESIS IN THE TREATMENT OF REFRACTORY ASCITES. A MULTICENTER RANDOMISED CONTROLLED STUDY

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Background and Aims: Refractory ascites (RA) develops in 10% of patients with advanced cirrhosis and is associated with poor prognosis. Management is large volume paracentesis (LVP) with albumin substitution. LVP severely affects quality of life, has a deleterious effect on nutrition, may be complicated by circulatory dysfunction and does not improve survival. TIPS (transjugular intrahepatic portosystemic shunt) is an alternative, but is associated with a high risk of hepatic encephalopathy and is contraindicated in advanced disease. A novel, implantable, programmable device (alfapump® system [AP] from Sequana Medical) moves ascites to the urinary bladder from where it is excreted naturally. The aim of this study is to compare AP and LVP in the management of RA in patients not suitable for TIPS.

Methods: Multi-center, open, RCT with RA & cirrhosis, randomised to AP or LVP. Follow-up is 12 months with a primary end-point of paracentesis-free survival (PFS). Secondary end-points include: cirrhosis-related complications, nutritional effects, quality of life, and survival. Exclusion criteria include: GI bleed ≤7 days, Serum Cr ≥2 mg/dl, platelet count <40,000, recurring SBP or UTI, loculated ascites, HCC (outside Milan criteria), and obstructive uropathy.

Data from a previous study (Pioneer) were used to estimate PFS for LVP and AP at 3 and 6 months post-implant. Sample size calculations were performed at a significance level of 5% and a power of 90%. Assuming a ratio of median PFS between groups is 2.5, for comparison at 3 months 60 subjects would be required, for comparison at 6 months 56 subjects would be sufficient.

Descriptive analysis will be performed for basal and post-basal measures. Variables will be described using means, medians, standard deviation, 95% confidence interval, minimum and maximum values. Changes will be analyzed using Student's t-test. In the case of variables with non-normal distribution, analysis will be done using nonparametric tests. The safety of the dispositive will be evaluated using a description of Serious Adverse Events.

Results: Table 1 summarises the trial activity across sites thus far.

Enrolment Target 60, Pre-screened 191, Consented 73, Screened 68, Screen Failures 14, Randomised 52, AP Group 24, LVP Group 27, Ongoing 18, Complete 10, Withdrawn 23, Lost to Follow-up 0.
Conclusions: The results of this trial are eagerly awaited and will provide the first randomised controlled evidence for the use of the AP as a possible treatment strategy for patients with RA.

P1320
EARLY EVEROLIMUS-BASED TACROLIMUS REDUCTION IN DE NOVO LIVER TRANSPLANT RECIPIENTS: DESIGN AND BASELINE DATA FROM HEPHAISTOS STUDY

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Background and Aims: Everolimus (EVR) might provide protection against viral replication, malignancy, and progression of fibrosis, apart from preventing nephrotoxicity by facilitating calcineurin inhibitor (CNI; tacrolimus [TAC]) reduction. Here we present the design and baseline data from the Hephaistos study evaluating the beneficial effects of early initiation of EVR in *de novo* liver transplantation recipients (LTxR).
Methods: Hephaistos is a 12-month (mo), multi-centre, open-label, controlled study aiming to randomise 330 *de novo* LTxR (1:1 ratio; 7–21 days post-transplantation) to receive EVR (C0 3–8 ng/mL) with reduced (r) TAC (C0 <5 ng/mL), or standard (s) TAC (C0 6–10 ng/mL) (Figure). Patients will receive induction therapy, mycophenolate mofetil, TAC, and corticosteroids as per local practice during run-in period. Randomisation is stratified by HCV status and lab MELD scores at transplantation. The primary objective of the study is to exhibit superior eGFR (MDRD-4 formula) with EVR+rTAC vs sTAC at month 12. Secondary objectives are to assess: the incidence of composite of treated biopsy proven acute rejection (tBPAR), graft loss, or death; the incidences of components of the composite efficacy endpoint; renal function by eGFR using various formulae (MDRD4, Nankivell, Cockcroft–Gault, CKD-EPI and Hoek formulae); incidence of proteinuria; incidence of AEs and serious AEs; incidence and severity of CMV and HCV infections and HCV-related fibrosis.

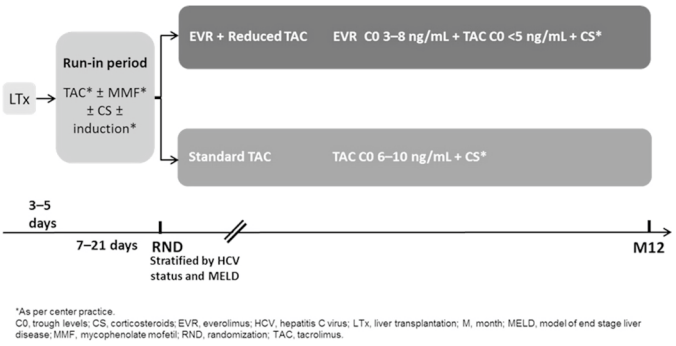

Figure: Hephaistos Study design.

Results: Hephaistos is an ongoing study recruiting LTxR at 15 centres across Germany. Currently 359 LTxR are screened and of these 156 patients are randomized (78 in each arm). At baseline, mean age (54.04 vs 54.21), male gender (77.8% vs 62.5%), Caucasian (100% vs 95.8%) and mean BMI (26.01 vs 26.81) were similar in EVR+rTAC and sTAC regimens respectively.

Conclusions: Hephaistos study aims to demonstrate benefits of early initiation of EVR+rTAC vs sTAC regimen by evaluating the renal function, efficacy, and safety particularly from recurrence of HCC, HCV and CMV.

P1321
A PHASE 3B, DOUBLE BLIND, PLACEBO CONTROLLED STUDY EVALUATING THE EFFECT OF OBETICHOLIC ACID ON CLINICAL OUTCOMES IN SUBJECTS WITH PRIMARY BILIARY CIRRHOSIS AT ELEVATED RISK OF PROGRESSION TO LIVER TRANSPLANT OR DEATH

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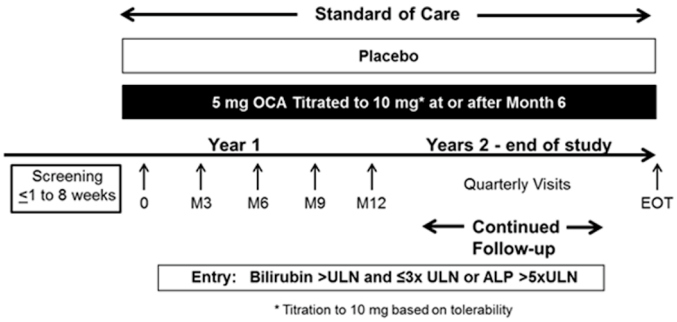
Background and Aims: Primary biliary cirrhosis (PBC) is an autoimmune cholestatic liver disease that leads to cirrhosis, transplantation and/or death. While transplant-free survival is the best demonstration of clinical benefit, slow disease progression makes this end point challenging. The hepatobiliary damage and secondary loss of function with PBC is reflected by increased plasma alkaline phosphatase (ALP) and bilirubin, respectively, which correlate with disease progression (Lammers 2014). Obeticholic acid (OCA) is in late-phase development for PBC. In the POISE trial after 12 months of OCA therapy, 46% [OCA Titration] and 47% [OCA 10 mg] of subjects met the primary composite endpoint of ALP <1.67×ULN *and* ≥15% ALP reduction *and* normal bilirubin compared to 10% of placebo subjects. This ongoing confirmatory Phase 3b study will evaluate OCA compared to placebo, standard of care continued, on major clinical outcomes.
Methods: 350 subjects with PBC at elevated risk for complications based on event rate predictions from the Global PBC group defined as ALP >5×ULN or bilirubin >ULN to ≤3×ULN will be randomized 1:1 to OCA or placebo. OCA dosing will initiate at 5 mg and after 6 months titrated to 10 mg based on tolerability. Given the inherent difficulties of a long-term placebo-controlled study in a rare disease both a placebo and a historical control are included. Total treatment duration is determined by the time to accrue the needed number of total primary events with a minimum 6 year follow-up expected. Should event rate accrual prove unfeasible due to challenges (enrollment or retention), randomization will stop and eligible subjects enrolled in open-label OCA treatment and comparisons made to matched historical controls.



EOT = end of treatment; M = month; UDCA = ursodeoxycholic acid; ULN = upper limit of normal

Figure 1. Schematic diagram, Study 747-302.

Results: The primary composite endpoint includes: death; liver transplant; model of end stage liver disease (MELD) score ≥15; hospitalization (defined by stay ≥24 hours) for new onset or

recurrence of: variceal bleed; encephalopathy; spontaneous bacterial peritonitis; uncontrolled ascites; hepatocellular carcinoma. 121 events provides 80% power to demonstrate a significant difference between OCA and placebo on time to liver-related outcomes, including all-cause mortality.
Conclusions: This study is designed to confirm the clinical benefit of OCA when added to standard of care. The design integrates a specific PBC patient population, event rate estimates, and end points from recent studies that correlate liver biochemistry with clinical outcomes.

P1322
CONTRAST (SONZOID)-ENHANCED US AS A SCREENING TOOL FOR HEPATOCELLULAR CARCINOMA IN CIRRHOSIS: AN EXPLORATORY CLINICAL TRIAL (SCAN TRIAL)

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Background and Aims: US has been used as the standard examination for HCC surveillance for it's noninvasiveness, convenience, and relatively lower price. However, the detection rate of early-stage HCC is low (63% of sensitivity), and more optimal surveillance tool is required to upgrade the detection rate while maintaining advantages of the US. Sonazoid, a recently developed new US contrast agent, enables not only the vascular phase imaging but also the Kupffer phase imaging with long-time window up to 3 hours. Several papers reported that Sonazoid is effective in characterizing focal liver lesions, grading histologic differentiation of HCC, guiding radiofrequency ablation or operation, and detecting small HCCs. However, it has not been generally implemented as a surveillance tool.
This study aimed to investigate the detection rate of early-stage HCC (BCLC 0/A) and the false referral rate when the Sonazoid contrast-enhanced US is used as a screening test in the high-risk group of HCC and to compare B-mode US and Sonazoid-US by intra-individual comparison.
Methods: This is a prospective, multi-center, explanatory, single group clinical trial to sound out the possibility prior to the fully randomized assessment study on the efficiency of the Sonazoid contrast-enhanced US in the HCC screening (NCT02188901). The patients fulfilling the following eligibility criteria will be included: (1) The high-risk group of the HCC scheduled to take the surveillance ultrasonography; (2) No prior HCC; (3) Cirrhosis related to HBV, HCV, and stage 4 primary biliary cirrhosis; (4) No contradiction to Sonazoid. The subjects will undergo the routine surveillance program (B-mode US) as planned and then kupffer phase image will be obtained between 10 and 30 minutes after the Sonazoid injection. The Liver US reporting and data system will be additionally prepared and implemented.
To obtain 80% statistical power with a 2-sided α equal to 0.05 and 10% drop-out rate, under the assumption of HCC incidence at very high risk group= 5%, sensitivity of G-US=63%, sensitivity of S-US=94%, 2262 patients per group are needed for a randomized controlled trial. In this exploratory trial, we will enroll 20% of S-US group (452 patients).
Results: In progress.
Conclusions: In progress.

P1323
A STUDY TO EVALUATE THE SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF REPEAT DOSES OF GSK2330672 ADMINISTRATION IN SUBJECTS WITH PRIMARY BILIARY CIRRHOSIS AND SYMPTOMS OF PRURITUS

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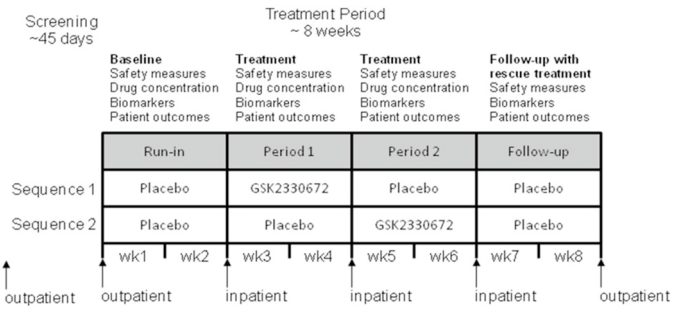
Background and Aims: Pruritus is a common symptom of cholestatic liver diseases such as primary biliary cirrhosis (PBC). There is a strong need for newer, safe and effective treatments for cholestatic pruritus. Bile acids have been proposed as potential pruritogens in cholestatic diseases. The ileal bile acid transporter (iBAT) expressed in the distal ileum, plays a key role in the enterohepatic circulation of bile acids. Pharmacological inhibition of iBAT is expected to interrupt enterohepatic recirculation, reduce bile acid concentrations in the liver and systemic circulation, resulting in improvement in pruritus.
The aims of this study are to administer oral GSK2330672 for 14 days to patients with PBC treated with ursodeoxycholic acid (UDCA) to: (1) investigate the safety and tolerability compared with placebo, (2) evaluate the effects on subjects' experience of pruritus, (3) evaluate effect on total serum bile acid concentrations and serum markers of bile acid synthesis and (4) study the effect on steady-state pharmacokinetics of UDCA. Additional exploratory analyses will be performed to identify potential biomarkers associated with PBC symptoms.
Study design: This is a UK multicentre, phase II, randomized, double-blind, placebo-controlled crossover trial recruiting patients with PBC and symptoms of pruritus. Patients must be on stable dose of UDCA or have demonstrated intolerance to UDCA. The total duration of subject participation will be up to 14 weeks. The participating centres include Newcastle, Birmingham and Cambridge.
The main inclusion criteria are: (1) male or female aged 18–75 years, (2) proven or likely PBC [based on serum alkaline phosphatase (AP), serology and/or liver biopsy], (3) screening AP value <10× upper limit of normal (ULN), (4) severe symptoms of pruritus that significantly impact daily life and have proven refractory after at least one previous therapy or unresolved symptoms with use of a single antipruritic agent or seeking treatment for pruritus that is newly diagnosed or previously untreated.



The Figure shows the study design. Eligible patients will participate in a 2-week placebo run-in period to assess compliance with study procedures and to record baseline symptoms on electronic diaries. Subjects will be randomized in a crossover fashion (Sequence 1/

Sequence 2) to receive placebo or GSK2330672 treatment during two consecutive 2-week study periods.
Sponsor: GlaxoSmithKline (GSK); BAT117213.
ClinicalTrials.gov Identifier: NCT01899703.

P1324
THE HOME STUDY (HOME MONITORING IN ENCEPHALOPATHY): RATIONALE AND STUDY DESIGN
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Background and Aims: Overt Hepatic Encephalopathy (HE) affects 30–45% of patients with cirrhosis. It is associated with severely impaired Health Related Quality of Life (HRQOL), likely impacted by the unpredictability of relapses and high hospital admission rates. First line treatment is usually with the non-absorbable disaccharide lactulose, however compliance with this drug can be poor. A system enabling home monitoring and remote management of HE has been developed as part of the EC FP7 project, d-LIVER. The HoME study is a pilot study of this system, primarily addressing patient acceptability.
Methods: A system was developed including software to be used by patients on a 10′ tablet-PC at home with wireless connectivity to the hospital. An electronic Number Connection Test (eNCT), based on the established paper NCT, and a stool frequency enquiry are carried out daily. Based on these parameters lactulose dosage is adjusted, if necessary. When there is evidence of deterioration despite appropriate treatment the health professional is alerted to take further action.
Results: Normative data for the eNCT showed a close correlation between the paper-based NCT and the eNCT (Pearson's 0.724, $p < 0.005$). The HoME study will include 20 patients aged 18 years or older with cirrhosis who have had an inpatient admission with overt HE, treated with lactulose. Patients are recruited while inpatients and require to have capacity to provide written informed consent. All patients undergo formal psychometric testing for HE at baseline [including the Psychometric Hepatic Encephalopathy Score (PHES)]. Patients are randomised to 6 months of management directed either by the new system or by the current standard of care (10 patients in each). Patients in the home monitoring group are asked to complete the eNCT and stool frequency enquiry daily with the primary outcome measure being patient acceptability at two, four and six months. Secondary outcome measures in the home monitoring group are completion rate and rate of change of therapy. In addition, HRQOL data (SF-36, SFLDQOL, PBC-40), health economics data, admission rates and HE recurrence rates are collected in both groups.
Conclusions: The HoME study evaluates the d-LIVER system for home monitoring in HE for the first time. The primary outcome is patient acceptability. Secondary outcomes, such as HRQOL and readmission rates, are unlikely to reach statistical significance in this study, but will inform the design of future larger scale trials of the system.

P1325
EFFICACY AND SAFETY OF COMBINED SEQUENTIAL TREATMENT WITH RFA AND SORAFENIB IN PATIENTS WITH HCC IN INTERMEDIATE STAGE INELIGIBLE FOR TACE: A PROSPECTIVE RANDOMIZED OPEN STUDY
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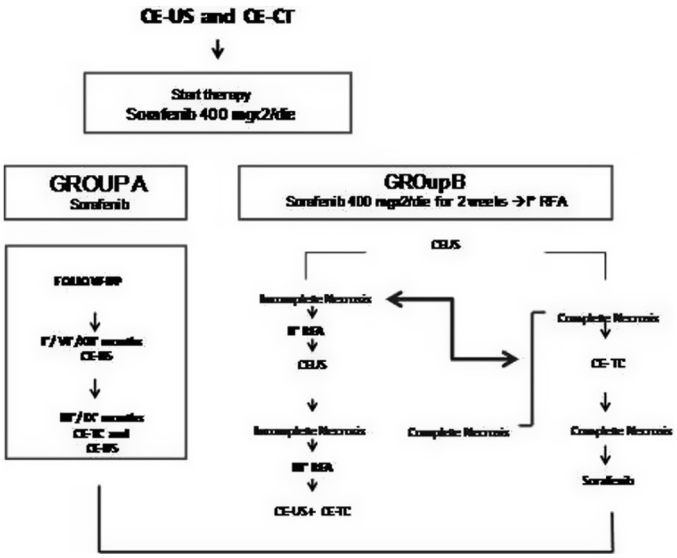
Background and Aims: TACE is the gold standard for intermediate hepatocellular carcinoma (BCLC-B, HCC). Patients (pts) who have contraindications or are not eligible to TACE are candidates for sorafenib. Aim of the present study is to verify the efficacy and safety of a combined treatment Sorafenib + radiofrequency ablation (RFA) in patients with intermediate HCC (BCLC-B) not eligible to TACE.
Methods: The present prospective randomized open-label study is expected to enroll 124 pts with BCLC-B, HCC (3–5 HCCs nodules ≥3 cm ≤5 cm), Child–Pugh A, age <80 years, not eligible to TACE or who refused TACE and were not eligible for surgical resection. Pts will be randomized 1:1 into two treatment arms: Group A, sorafenib (800 mg/day); Group B, combined sequential treatment sorafenib + RFA. Group B will start sorafenib for 2 weeks; then sorafenib is stopped from the 15th to the 19th day, to allow RFA scheduled on the 17th day. Pts will undergo CEUS to assess the extent of necrosis and withdrawals chemistry 24 hours after RFA. In case of incomplete necrosis, two days after RFA, pts will start again sorafenib at full dose for 11 days; then suspend the molecule two days before the II sitting RFA and resume it after only two days from the procedure itself (possible up to 3 sessions of RFA, in each session will be treated maximum 2 nodules or a single nodule up to 5 cm size). Seven days after the last RFA therapeutic efficacy will be evaluated with CEUS and three-phase contrast-enhanced (CE) spiral CT. In case of complete necrosis, pts will resume sorafenib two days after the RFA. The therapeutic efficacy will be evaluated in Group A and in Group B with CE-CT and CE-US 3 months after the start of treatment. The primary endpoint was overall survival. Secondary objectives: validation of CEUS in the evaluation of treatment efficacy RFA; safety and efficacy of the combination Sorafenib + RFA in pts with HCC BCLC-B. Sample size has been calculated by assuming a proportion of survival after 16 months of 50% in arm A and 75% in arm B, with a predicted percentage of drop-out of 5%. The time of expected enrollment is 12 months to make a comparison with the log rank test, with an α error of 0.05 and a power of 80%. Forty events are needed equal to a total sample size of 124 patients (62 patients per treatment group). Randomization will be performed with a list of *a priori* sample prepared in sealed envelopes opaque.
EuDRACT number: 2014–003925–16. Protocol Code Number: 054.



P1326

PASIREOTIDE IN ADDITION TO ASPIRATION SCLEROTHERAPY TO IMPROVE TREATMENT OF LARGE SYMPTOMATIC HEPATIC CYSTS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Background and Aims: Aspiration sclerotherapy (AS) is a therapeutical option for large symptomatic hepatic cysts. However, inadequate cyst reduction following AS is frequently reported. Strong post-procedural cyst fluid secretion by cholangiocytes, lining the epithelium of the hepatic cyst, seems to be associated with lower reduction rates. Previous studies showed that somatostatin analogues curtail cyst cholangiocyte fluid production. We hypothesize that combining the long-acting somatostatin analogue pasireotide (SOM230) with AS will decrease post-procedural fluid reaccumulation resulting in more effective hepatic cyst reduction.

Methods: This is a single center, randomized, double-blind, placebo-controlled clinical trial. Patients with a large (>5 cm) symptomatic hepatic cyst are eligible for inclusion. A total of 34 patients will be randomized in a 1:1 ratio. All patients will undergo AS, comprising percutaneous cyst aspiration followed by instillation of 100% ethanol (ratio of 10%, never exceeding 50 mL) for 10 minutes. In the active arm, patients receive a pasireotide 60 mg injection two weeks prior and two weeks following AS. Controls receive placebo injections at corresponding intervals. Primary endpoint is the proportional cyst diameter reduction four weeks after AS measured by ultrasonography. Diameter reduction at 12 and 24 weeks after AS will be measured as secondary outcomes. Symptomatic relief will be assessed with a standardized, 7-point scale questionnaire and health-related quality of life will be evaluated using the generic short-form health survey (SF-36). Finally, safety and tolerability will be assessed.

Results: Registration: ClinicalTrials.gov: NCT02048319, January 6, 2014; EudraCT: 2013–003168–29, August 16, 2013

Conclusions: In this trial we investigate the additional effect of pasireotide to AS. By curtailing post-procedural cholangiocyte fluid reaccumulation we aim to optimize efficacy of cyst regression ultimately leading to better symptomatic relief and reduced recurrence rates.

P1327

ATTIRE: ALBUMIN TO PREVENT INFECTION IN CHRONIC LIVER FAILURE

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Background and Aims: Patients with cirrhosis are at a greatly increased risk of severe bacterial infection with survival directly related to extra-hepatic organ dysfunction. A defective innate immune response is considered to underlie this risk. There is however no medical strategy to restore immune competence in these patients.

Elevated circulating Prostaglandin E_2 (PGE_2) levels contribute to immune suppression in acutely decompensated (AD) cirrhosis. PGE_2 is more bioavailable because of decreased serum albumin levels in AD patients as albumin binds PGE_2. The binding capacity of endogenous albumin is also known to be defective in cirrhosis. Human Albumin Solution (HAS), a safe and common intervention, could thus be repurposed as an immune restorative drug in AD patients.

The primary aim of ATTIRE is to determine if raising serum albumin to >30 g/L in patients with decompensated cirrhosis will decrease rates of infection, extra hepatic organ dysfunction and death.

Methods: All patients admitted to hospital with acute onset or worsening of complications of cirrhosis, 18–75 years old, predicted hospital admission >5 days, serum albumin <30 g/L, randomised within 72 hours of hospital admission.

Exclusion Criteria: HIV, advanced HCC, predicted survival <48 hrs, severe cardiac dysfunction.

Stage 1 is a feasibility study (n = 80). All patients will be given daily 20% HAS according to a suggested protocol dependent on serum albumin. This will determine if patient's serum albumin can be maintained at >30 g/L.

Stage 2 is a multicenter open label randomised controlled trial (n = 880). Patients will be randomised to daily 20% HAS to target a serum albumin of 35 g/L OR standard of care for maximum of 14 days.

The composite primary endpoint within the intervention period has 3 components:

1. Noscomial Infection indicated by any new (or change in) prescribed antibiotics.
2. Extra Hepatic Organ Dysfunction.
   - RENAL: Serum creatinine increases by ≥50% compared to randomization value
   - CEREBRAL: Development of grade III or IV encephalopathy (Westhaven criteria)
   - CIRCULATORY: Decrease in MAP to <60 mmHg or initiation of inotropic support
   - RESPIRATORY: any single point increase in SpO_2/FiO_2 ratio in defined criteria
3. Death.

Conclusions: ATTIRE is a large UK multicenter RCT that will evaluate the impact of albumin on rates of infection, extrahepatic organ dysfunction and death in AD patients. EudraCT 2014-002300-24. Visit www.attiretrial.com for more information.

P1328

RITPBC: B-CELL DEPLETING THERAPY (RITUXIMAB) AS A TREATMENT FOR FATIGUE IN PRIMARY BILIARY CIRRHOSIS: STUDY PROTOCOL FOR A RANDOMISED CONTROLLED TRIAL

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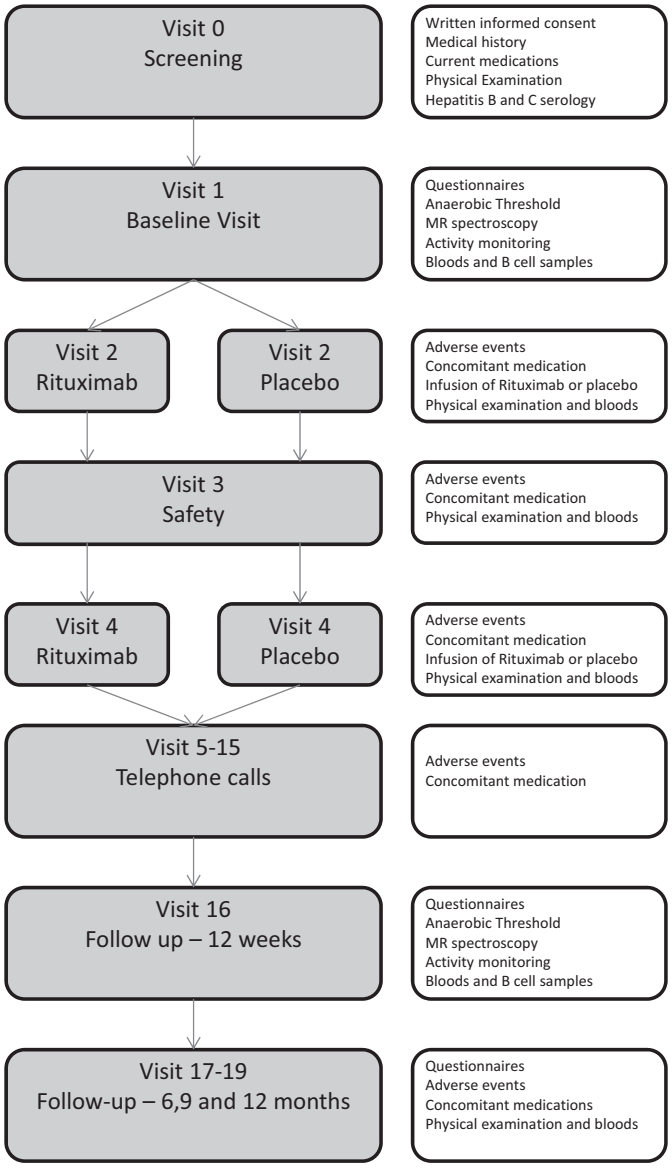
Background and Aims: Primary Biliary Cirrhosis (PBC) is an autoimmune liver disease with 45% of patients experiencing clinically significant fatigue. Fatigue often has a negative impact on quality of life, particularly when it results in social isolation. Fatigue is highlighted by patients as a priority for research and patient support groups were involved in the design and development of this trial. Mechanistic studies have led to the hypothesis that AMA and its activity against PDC results in muscle bio-energetic abnormalities by over-utilisation of anaerobic pathways. B-cell depleting agents could therefore be a potential treatment for fatigue in PBC. Pilot studies of Rituximab have shown an improvement in fatigue but were powered for biochemical endpoints rather than symptoms.

Methods: RITPBC is an MRC and NIHR Efficacy and Mechanism Evaluation Programme (EME) funded project. It is a phase II, single-centre, randomised controlled, double blinded trial comparing Rituximab with placebo in fatigued Primary Biliary Cirrhosis patients. 78 patients with PBC and clinically significant fatigue will be randomised to receive two infusions of Rituximab or placebo. Fatigue severity will be assessed by the PBC-40, a patient-derived, disease specific, fully validated, quality of life measure.

The trial aims to assess whether Rituximab improves fatigue in PBC, the safety and tolerability and the sustainability of any beneficial actions. The primary outcome will be an improvement in fatigue

domain score of the PBC-40 evaluated at 12 weeks. Secondary outcome measures include novel MRI techniques to assess muscle bioenergetic function, physical activity, anaerobic threshold, liver biochemistry and quality of life measures.

Conclusions: This is a landmark trial as it is the first randomised controlled trial to investigate a therapeutic agent as a treatment for fatigue in PBC. The protocol describes a model for fatigue trials including trial endpoints and sample size calculations which will be transferrable to future trials of fatigue both in PBC and in other disease areas. The trial protocol is innovative as it utilises novel MRI techniques as an outcome measure. Recruitment strategies utilising the UK-PBC MRC trials platform have been implemented in RITPBC, these methods have the potential to revolutionise the way trials are conducted in rare diseases. Recruitment started in October 2012 and to date, 38 patients have been recruited, making it the largest biologicals trial in liver disease.



P1329

A PLACEBO CONTROLLED SINGLE CENTRE DOUBLE BLIND RANDOMISED TRIAL TO INVESTIGATE THE EFFICACY OF RIFAXIMIN IN IMPROVING SYSTEMIC INFLAMMATION AND NEUTROPHIL MALFUNCTION IN PATIENTS WITH CIRRHOSIS AND CHRONIC HEPATIC ENCEPHALOPATHY ('RIFSYS')

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Background and Aims: Hepatic encephalopathy (HE) is a devastating complication of cirrhosis which portends a poor prognosis without liver transplantation. It results from a dysfunctional gut–liver–brain axis and historically therapies have primarily targeted gut ammonia metabolism. The non-absorbable gut-selective antibiotic rifaximin-α is efficacious in chronic HE reducing recurrent overt episodes and improving cognitive function in minimal HE. Rifaximin-α has broad antimicrobial spectrum and may inhibit division of urea-deaminating bacteria, thereby reducing gut ammonia production. However, recent studies have failed to demonstrate a convincing reduction in blood ammonia and the specific mechanism of action of rifaximin-α has yet to be elucidated. Neutrophil dysfunction is recognised in cirrhosis as predisposing to infection and organ dysfunction, and may also have a more direct pathogenic role in HE. A therapeutic strategy utilizing rifaximin-α to modulate gut bacteria could potentially lower gut-derived systemic inflammation, endotoxemia, neutrophil dysfunction, and organ dysfunction improving outcomes and prolonging transplant-free survival.

Methods: An investigator-initiated randomised placebo-controlled single centre double blind study [ClinicalTrials.gov ref: NCT 02019784] has therefore been designed to examine the impact of rifaximin-α 550 mg (TARGAXAN) bid for 90 days on parameters of immune and gut dysfunction in patients with liver cirrhosis experiencing chronic HE. Patients with cirrhosis presenting with chronic persistent overt HE (≥ grade 1) or with ≥2 episodes of overt HE in the previous 6 months will be recruited.

Primary end point: To test if rifaximin-α reduces neutrophil spontaneous oxidative burst *ex vivo* in patients with cirrhosis and chronic HE after 30 days.

Secondary end points: To test if rifaximin-α reduces the development of systemic inflammation, organ failure and improves survival over 90 days. This will include analyses for intestinal permeability and microbiota, alterations in faecal biomarkers, plasma and urine metabolic profiling, markers of bacterial translocation (blood bacterial DNA quantification) and neutrophil dysfunction (including toll-like receptor 4 expression). 50 participants will be randomised on 1:1 basis to either Rifaximin-α or placebo with 90 days treatment duration with assessments at baseline (day 0) prior to initiation of medication, 30 and 90 days.

Results: Trial recruitment closes Nov 2015 with anticipated publication of data mid-2016.

P1330

HIGH DOSE STEROIDS VERSUS INTRAVENOUS IMMUNOGLOBULIN PLUS STEROIDS IN OPERATED BILIARY ATRESIA PATIENTS

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Introduction: Biliary atresia (BA) is the most prevalent cause of liver-related death in children and the most frequent indication for liver transplantation in the pediatric population. Recently, Davenport et al. found that the use of prednisolone significantly improved early post-operative liver biochemistry (especially at the higher dose), and increased the proportion of infants who cleared their jaundice at 6 months post-KPE.

The actual primary target(s) of IVIG in autoimmune disease are still unclear. IVIG may work via a multi-step model where the injected IVIG first forms a type of immune complex in the patient (Clynes, 2005). Once these immune complexes are formed, they interact with activating Fc receptors on dendritic cells which then mediate anti-inflammatory effects helping to reduce the severity of the autoimmune disease or inflammatory state (Siragam et al., 2006). IVIG may also regulate the immune response by reacting with a number of membrane receptors on T cells, B cells, and monocytes *that are pertinent to autoreactivity and induction of tolerance to self* (Bayry et al., 2003).

Aim: Evaluating the effect of administrating IV IG plus high dose steroids post-KP on the outcome of operated BA patients and comparing it with the use of post-KP high dose steroids alone.

Methods: The study population consists of two main groups:

Group A: This group will include 30 post-KP BA patients and this group will be given oral prednisolone on the following regimen: On day 5–9 postoperative, prednisolone 5 mg/kg/day. On day 10–14 postoperative, prednisolone 4 mg/kg/day. On day 15–19 postoperative, prednisolone 3 mg/kg/day. On day 20–24 postoperative, prednisolone 2 mg/kg/day. On day 25–29 postoperative, prednisolone 1 mg/kg/day. On day 30–32 postoperative, hydrocortisone 2.5 mg/kg/dose twice daily. On day 33–35 postoperative, hydrocortisone 2.5 mg/kg/dose once daily.

Group B: This group will include 30 post-KP BA patients and this group will be given oral prednisolone on the same previous regimen plus two doses of IV IG 1 g/kg/dose on day 1 and 30 postoperative.

According to the one year postoperative outcome, patients will be classified into two groups: good outcome group (well colored stool and total bilirubin <2.0 mg/dL) and bad outcome group (persistent clay-colored or pale stool, rapidly progressive disease, the incidence of death due to the liver disease or total bilirubin ≥2 mg/dL).

P1331

PHASE 3B STUDIES TO ASSESS LONG-TERM CLINICAL OUTCOMES IN HCV GT1-INFECTED PATIENTS TREATED WITH OMBITASVIR/PARITAPREVIR/RITONAVIR AND DASABUVIR WITH OR WITHOUT RIBAVIRIN

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Background and Aims: Curing HCV infection has been demonstrated to reduce the risk of progression to cirrhosis, liver decompensation, hepatocellular carcinoma (HCC), and mortality. The objectives of these phase 3b studies are to evaluate the effect of sustained virologic response at post-treatment week 12 (SVR12) on the progression of liver disease for 5 years following treatment with the interferon (IFN)-free three direct-acting antiviral (3D) regimen of ombitasvir/paritaprevir (identified by AbbVie and Enanta, co-administered with ritonavir) and dasabuvir with or without ribavirin (RBV) in HCV genotype (GT) 1-infected patients.

Methods: TOPAZ-I and -II are phase 3b, open-label, non-randomized, multicenter trials will enroll approximately 2250 patients (pts): 1650 pts from about 200 sites representing 29 countries outside the United States (TOPAZ-I; NCT02219490), and approximately 600 pts from roughly 50 sites in the United States (TOPAZ-II; NCT02167945). Study participants will include HCV GT1-infected pts with or without prior IFN/RBV treatment experience, including pts with compensated cirrhosis. Patients with GT1b infection and no cirrhosis will receive the 3D regimen without RBV for 12 weeks; GT1b pts with cirrhosis will receive 3D+RBV for 12 weeks. Patients with GT1a infection without cirrhosis and GT1b pts with cirrhosis will receive 3D+RBV for 12 weeks. Patients with GT1a infection will be treated with 3D+RBV for 12–24 weeks. Broad inclusion criteria will allow enrollment of pts with no upper limit on age, BMI, or ALT/AST levels and include pts with creatinine clearance ≥30 mL/min, albumin ≥2.8 g/dL, and platelet count ≥25,000 cells/mm^3.

TOPAZ-I and -II share a primary endpoint evaluating the effect of SVR12 on the incidence of all-cause death, liver-related death, liver decompensation, liver transplantation, HCC, and the composite of any of these clinical outcomes. Additional endpoints include: the percentage of pts achieving SVR12 for each study ITT population; long-term liver fibrosis progression measured by transient elastography (TOPAZ-I only); mean change from baseline in patient-reported quality of life and fatigue assessed by the SF-36v2 survey and FACIT-F questionnaire, respectively; and adherence to study drugs during the treatment period (TOPAZ-II only). Safety and tolerability will be assessed by monitoring adverse events, physical examinations, clinical laboratory tests, and vital signs.

P1332

THE ASTRAL STUDIES: EVALUATION OF SOF/GS-5816 SINGLE TABLET REGIMEN FOR THE TREATMENT OF GENOTYPE 1–6 HCV INFECTION

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Background and Aims: GS-5816 is a second-generation NS5A inhibitor that has potent in vitro antiviral activity across all HCV genotypes. The co-administration of sofosbuvir (SOF) and GS-5816 for 12 weeks without ribavirin (RBV), was well-tolerated and resulted in high SVR12 in patients with genotype 1–6 HCV infection in Phase 2 studies.

Methods: The Phase 3 ASTRAL program comprises 4 clinical studies evaluating the safety and efficacy of treatment with a fixed dose combination (FDC) of SOF/GS-5816 (400 mg/100 mg) in patients with genotype 1–6 HCV infection, including including those with prior treatment failure and cirrhosis, and also patients with decompensated cirrhosis.

Results: The ASTRAL-1 study (NCT02201940) is a double-blind, placebo-controlled study that will randomize 600 treatment-naïve and treatment-experienced patients with and without compensated cirrhosis with genotype 1–6 HCV infection in a 5:1 ratio to SOF/GS-5816 or placebo for 12 weeks. The study is being conducted at 81 sites in Belgium, Canada, France, Germany, Hong Kong, Italy, United Kingdom and the United States.

The ASTRAL-2 study (NCT02220998) is an open-label, active-comparator study that will randomize 240 treatment-naïve and treatment-experienced patients with and without compensated cirrhosis with genotype 2 HCV infection (1:1) to SOF/GS-5816 for 12 weeks or SOF+RBV for 12 weeks. The study is being conducted at 51 sites in the United States.

The ASTRAL-3 study (NCT02201953) is an open-label, active-comparator study that will randomize 500 treatment-naïve and treatment-experienced patients with and without compensated cirrhosis with genotype 3 HCV infection (1:1) to SOF/GS-5816 for 12 weeks or SOF+RBV for 24 weeks. The study is being conducted at 76 sites in Australia, Canada, France, Germany, Italy, New Zealand, United Kingdom and the United States.

The ASTRAL-4 study (NCT02201901) is an open-label study that will randomize 225 patients with genotype 1–6 HCV infection with Child–Pugh–Turcotte (CPT) class B cirrhosis (1:1:1) to SOF/GS-5816 for 12 weeks, SOF/GS-5816+RBV for 12 weeks or SOF/GS-5816 for 24 weeks. The study is being conducted at 50 sites in the United States.

Conclusions: The ASTRAL clinical program will evaluate SOF/GS-5816 in more than 1000 patients with genotype 1–6 HCV infection including patients with advanced liver disease. If successful, SOF/GS-5816 will provide a simple, highly effective, well tolerated, pangenotypic single tablet regimen (STR) for the treatment of HCV infection globally.

P1333
A RANDOMIZED, DOUBLE-BLIND STUDY OF THE EFFECTS OF OMEGA-3 FATTY ACIDS (OMEGAVEN™) ON OUTCOME AFTER MAJOR LIVER RESECTION

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Background and Aims: The body is dependent on the exogenous supply of omega-3 polyunsaturated fatty acids (n3-PUFA). These essential fatty acids are key players in regulating metabolic signaling but also exert anti-inflammatory and anti-carcinogenic properties. The liver is a major metabolic organ involved in fatty acid metabolism. Under experimental conditions n3-PUFA exert beneficial effect on hepatic steatosis, regeneration and inflammatory insults such as ischemic injury after surgery. Some of these effects have also been observed in human subjects. However, it is unclear, whether perioperative application of n3-PUFA is sufficient to protect the liver from ischemic injury. Therefore, we designed a randomized controlled trial (RCT) assessing n3-PUFA (pre-) conditioning strategies in patients scheduled for liver surgery.

Methods: The Omegaven™ trial is a multi-centric, double-blind, randomized, placebo-controlled trial applying two single doses of Omegaven™ or placebo on 258 patients undergoing major liver resection. Primary endpoints are morbidity and mortality one month after hospital discharge, defined by the Clavien-Dindo classification of surgical complications as well as the Comprehensive Complication Index (CCI). Secondary outcome variables include length of Intensive Care Unit (ICU) and hospital stay, postoperative liver function tests, fatty acid and eicosanoid concentration, inflammatory markers in serum and in liver tissue. An interim analysis is scheduled after the first 30 patients.

Conclusions: Long-term application of n3-PUFA have a beneficial effect on metabolism and hepatic injury. Patients often require surgery without much delay, thus long-term n3-PUFA uptake is not possible. Also, lack of compliance may lead to incomplete n3-PUFA substitution. Hence, perioperative application of Omegaven™ may provide an easy and controllable way to ensure hepatic protection.

P1334
A MULTICENTRE, RANDOMISED, OPEN-LABEL STUDY COMPARING THE EFFICACY AND SAFETY OF TWO DOSES OF DOXORUBICIN TRANSDRUG™ TO BEST STANDARD OF CARE IN PATIENTS WITH ADVANCED HEPATOCELLULAR CARCINOMA (HCC) AFTER SORAFENIB. THE RELIVE STUDY

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Background and Aims: Doxorubicin Transdrug (DT) is a nanoparticular formulation of doxorubicin shown preclinically to bypass the multidrugresistance to chemotherapy and improve the biodistribution of doxorubicin into the liver and tumor cells. In a previous study in HCC patients, DT was shown to increase overall survival but pulmonary toxicity prevented its use by intraarterial route. DT is currently under evaluation using the IV route in a phase III trial in patients previously progressive under sorafenib (NCT01655693).

Methods: The ReLive study is a randomized, 3 parallel arms, open-label study with stratification on geographic regions. Patients with advanced HCC having received at least one prior treatment including sorafenib, with Child–Pugh A to B7 cirrhosis, ECOG <2

Table (abstract P1333).

Sponsor investigator	Prof. Pierre-Alain Clavien, MD PhD
Study product	Omegaven™, 100 ml intravenously administered at the evening before and during liver resection.
Primary endpoint	Postoperative morbidity and mortality determined by the Clavien–Dindo classification of surgical complications and the Comprehensive Complication Index (CCI) 1 month after hospital discharge.
Secondary endpoints	
Main center	Serum samples: postoperative peak AST and ALT, fatty acids and n3-PUFA concentration, inflammatory markers. Liver biopsy: histology (necrosis, apoptosis), inflammatory markers, hepatic fatty acid and n3-PUFA content
Main center and external centers	Duration of hospitalization and ICU stay. Hematology: hemoglobin, hematocrit, leukocytes, platelets, INR. Chemistry: triglycerides, bilirubin, AST, ALT, ALKP, creatinine, CRP
Methodology	Randomized, double-blind, placebo controlled
Clinical phase	Phase III (new indication for Omegaven™)
Study duration	3 years (start July 2013)
Study centers	Multi-center (Zurich, Bucharest, Moscow)
Number of subjects	258 patients
Main inclusion criteria	Adults (over 18 years) requiring liver resection of at least 1 segment or multiple wedge resections (≥3); no coagulopathy (INR ≤1.2, platelets ≥150,000×10^3/μl)
Main exclusion criteria	Liver resections <1 segment, wedge resections (<3); coagulopathy (INR >1.2, platelets <150,000×10^3/μl); hypertriglyceridemia (>5.0 mmol/l); liver cirrhosis; severe renal failure (estimated GFR <30 ml/min/1.73 m^2); pregnancy.
ClinicalTrials.gov	ID: NCT01884948

and adequate cardiac and pulmonary functions are eligible.
Patients receive DT 20 mg/m^2, DT 30 mg/m^2 or best standard of care (BSC). DT is administered through a slow 6 hours intravenous infusion every 4 weeks until progression or toxicity. In control group, patients are treated according to centre's usual practice.
Primary endpoint is overall survival (OS). Main secondary efficacy endpoints are response rate and progression-free survival according to RECIST 1.1 and centralized independent radiologic review. A pharmacokinetic study is performed in selected centers. An independent data safety monitoring board (DSMB) blindly reviews safety data every 6 months.
To demonstrate an OS superiority in comparing each DT groups versus BSC with a hazard ratio of 0.6, a sample size of 130 patients/group has been calculated with 90% power.
The study was initially conducted in limited centers in France to evaluate the safety of this new administration schedule. Safety analyses did not show pulmonary toxicity. The study was thus extended to Italy, Spain, Germany, Belgium, Austria, Hungary, USA and Russia. Additional sites in Australia, Turkey, Lebanon, Egypt and KSA will be open in 2015.
The first patient was included in June 2012. By mid December 2014, 136 patients have been randomized and 289 DT infusions administered in 75 patients. The last DSMB review recommended continuing the study without any modification.
Conclusions: Since approval of sorafenib, no other treatment has demonstrated a significant OS improvement. The aim of this phase 3 study is to evaluate whether a nanoformulation of systemic chemotherapy (doxorubicin) can improve the OS of advanced HCC patients after failure to one or more prior tyrosine kinase inhibitor treatments.

P1335

A RANDOMIZED, CONTROLLED PHASE III TRIAL OF SORAFENIB WITH OR WITHOUT CONVENTIONAL TRANSARTERIAL CHEMOEMBOLIZATION IN PATIENTS WITH ADVANCED HEPATOCELLULAR CARCINOMA (STAH)

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Background and Aims: The conceptual advantage of a combination of transarterial chemoembolization (TACE) plus anti-angiogenic agents is the possible synergistic effect in patients with hepatocellular carcinoma (HCC). Positive results of two phase II studies combining TACE and sorafenib have been reported. The COTSUN study (NCT00919009) showed a promising time to progression in patients with advanced HCC. However, no study shows the additional effect of TACE with sorafenib treatment in patients with advanced HCC, particularly in patients with macrovascular invasion or extrahepatic metastasis.
Methods: STAH is a randomized, multi-center, open-labeled, phase III study in patients with advanced HCC. A total of 338 patients will be randomized (1:1) into 1 of the 2 treatment arms: sorafenib with (arm combination treatment; arm C) or without (arm sorafenib alone; arm S) conventional TACE (cTACE). Randomization will be stratified according to the modified International Union Against Cancer (mUICC) stage (III vs. IV), vascular invasion (none and Vp1–2 vs. Vp3–4 and any other presence), Child–Pugh score (5 vs. 6–7), and serum alpha-fetoprotein level (≥200 ng/mL vs. <200 ng/mL). The primary objective is to determine overall survival (OS) in the 2 arms. Assuming a median OS associated with sorafenib treatment of 9.5 months and a median OS associated with combination treatment of 13.0 months, 241 events will provide an 80% one-sided power with an α value of 0.05 to detect a difference in OS between the 2 groups.
The main inclusion criterion is advanced HCC (mUICC stage III, IVa, IVb) that is indicated for systemic chemotherapy as the treatment of choice according to the Korean guidelines. All eligible patients will take sorafenib (both arms) within 3 days after randomization, while patients in arm C will undergo the first cTACE within 14 days after randomization, with subsequent cTACE performed as needed according to re-determined criteria. Enrollment has begun February 2013 and two-hundred two patients (59.8% of the target population) have been enrolled in this study by 30 November 2014.
Clinical trial information: NCT01829035.

P1336

TREATMENT OF TYPE 1 HEPATORENAL SYNDROME WITH TERLIPRESSIN INFUSION ADJUSTED ACCORDING TO HEMODYNAMIC RESPONSE. THE AMELIORATE STUDY

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Background and Aims: First-line treatment for type 1 hepatorenal syndrome (HRS) is currently based on terlipressin administered as intravenous bolus associated with albumin. There are data showing a direct relationship between increase in mean arterial pressure (MAP) during treatment and response to therapy. However, terlipressin dose is adjusted according to changes in serum creatinine. Recent studies suggest that terlipressin administered as continuous infusion could be more effective and with less side effects. The hypothesis of the study is that terlipressin administred as continuous infusion and with an early dose adjustment according to hemodynamic response could improve the efficacy and safety of treatment in type 1 HRS.
Endpoints: Primary endpoint is to evaluate the effects of treatment with terlipressin infusion with dose adjusted according to hemodynamic response in type 1 HRS. Secondary endpoints are: to identify predictive factors of response to therapy and assess adverse events.
Study design: Proof-of-concept, prospective, multicenter study that will include 40 patients with cirrhosis and type 1 HRS. Exclusion criteria are hepatocellular carcinoma beyond Milan criteria, advanced extrahepatic diseases or contraindications to receive terlipressin. Patients will receive terlipressin administered as continuous infusion and albumin at standard doses. Dose of terlipressin will be adjusted according to hemodynamic response (increase ≥10 mmHg in MAP with respect to pretreatment value) and to changes in serum creatinine. Initial terlipressin dose is 2 mg/day. MAP will be monitored every 8 h and serum creatinine will be determined daily. Terlipressin dose will be increased 2 mg every 8 h until an increase in MAP >10 mmHg with respect to pretreatment value or a decrease >25% in serum creatinine with respect to previous value are achieved. Maximum dose of terlipressin is 12 mg/day. Treatment will be maintained until resolution of type 1 HRS or maximum of 14 days. Complete response will be defined as a decrease in serum creatinine <1.5 mg/dL. Partial response will be defined as a 50% reduction in serum creatinine with final value >1.5 mg/dL. Liver and circulatory function (renin, noradrenaline) will determined at baseline and days 3 and 7.

Urinary biomarkers will be measured and analyzed for their relationship with response to therapy. The development of adverse events will be recorded.

P1337

RANDOMIZED TRIAL WITH RIFAXIMIN IN LIVER CIRRHOSIS. EFFECTS ON THE HAEMODYNAMIC AND INFLAMMATORY STATE

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Background and Aims: Bacterial infections occur frequently in cirrhosis, often triggering complications to decompensated cirrhosis. Bacterial translocation from the gut has been hypothesized to worsen the haemodynamic derangement and trigger infections in the cirrhotic patient and affect the risk of complications to cirrhosis. Rifaximin is applied in the treatment and prophylaxis of hepatic encephalopathy and known to affect both aerobe and anaerobe bacteria in the intestinal lumen. In this trial we aim to investigate the pathophysiological mechanisms of rifaximin on the bacterial translocation and evaluate the effects on splanchnic and systemic haemodynamics and inflammation in the cirrhotic patient.
Methods: Between January 2013 and December 2015 a randomized, double blind, placebo controlled trial will be performed. Inclusion criteria are cirrhotic Child-Turcotte class B or C patients and ascites present on ultrasound within the past three months and a hepatic venous pressure gradient (HVPG) above10 mmHg. Exclusion criteria are Child-Turcotte score above 12, infection, renal failure, and overt hepatic encephalopathy. The patients will be randomized to receive either oral rifaximin, 1100 mg/day or identical placebo for 28 days. The investigational programme includes measurements of HVPG, cardiac output, plasma volume, glomerular filtration rate (GFR) as measured by Cr-51-EDTA, full biochemical evaluation, continuous reaction times and psychometric hepatic encephalopathy score. All measurements will be performed at baseline and repeated at day 29. Patients are followed for at least six months, until death or end of trial. Data will be compared over time and between groups. Rates of complications and survival will be calculated. Primary outcome measures are effect on HVPG and GFR. Secondary outcome measures include, but are not limited to, effect on systemic vascular resistance, haemodynamic and inflammatory markers, biochemistry, state of hepatic encephalopathy, complication rates and survival.
Results: Data will be analyzed January 2016.
Conclusions: Until now there are no conclusive data on the effects of rifaximin on the haemodynamics and the inflammatory state in cirrhosis. We expect that the results of the present trial will significantly contribute to reveal these important clinical questions.

P1338

AN INTERNATIONAL OBSERVATIONAL STUDY TO ASSESS THE USE OF SORAFENIB AFTER TRANSARTERIAL CHEMOEMBOLIZATION (TACE) IN PATIENTS WITH HEPATOCELLULAR CARCINOMA (HCC): OPTIMIS

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Background and Aims: TACE is currently recommended for the treatment of patients with intermediate-stage HCC (Barcelona Clinic Liver Cancer [BCLC] stage B). However, it remains unclear which patients are most likely to benefit from TACE, or when TACE should be stopped and alternative treatments considered. Currently there is no globally accepted clinical algorithm for TACE use. The multikinase inhibitor sorafenib is the only systemic therapy currently approved for the treatment of unresectable HCC.
Methods: Patients aged ≥18 years, with histologically/cytologically documented or radiographically diagnosed HCC classified as BCLC stage B or higher, with a life expectancy of ≥8 weeks, and for whom a decision to treat with TACE has been made at the time of study enrollment (one prior TACE treatment is allowed if performed at the same center and all required data about the procedure are available), are eligible. Treatment decisions made before a patient is enrolled, and during the observation period, must be according to investigators' regular practice. Exclusion criteria include any systemic anti-cancer therapy prior to the first TACE, or participation in an interventional study of locoregional or systemic therapy. The primary objective is evaluation of overall survival in patients who received sorafenib subsequent to TACE non-eligibility (early sorafenib) compared with those who did not (non-early sorafenib). The non-early sorafenib group includes patients who did not receive sorafenib or who received it at a later point. Secondary objectives include progression-free survival, time to progression, tumor response, and safety. Planned enrollment is approximately 1600 patients from about 30 countries across Europe, Latin America, and Asia-Pacific, and from Canada. One interim analysis is planned once 500 patients have been observed for at least 6 months. Final analysis will be performed once the last enrolled patient has been followed for 18 months, has been lost to follow-up, or has died. The study began in October 2013 and 737 patients have been enrolled as of November 2014.
ClinicalTrials.gov identifier: NCT01933945.

P1339

EVALUATING THE EFFICACY AND SAFETY OF EVEROLIMUS WITH REDUCED TACROLIMUS VERSUS STANDARD TACROLIMUS IN LIVING DONOR LIVER TRANSPLANT RECIPIENTS: H2307 STUDY DESIGN

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Background and Aims: Living donor liver transplantation (LDLT) is a valuable option for mitigating the shortage of organs. Little data exists from randomised clinical studies evaluating the effect of different immunosuppressive regimens in LDLT recipients.

H2307 is the first study to evaluate the efficacy and safety of everolimus (EVR) + reduced tacrolimus (rTAC) vs standard TAC (TAC-C) in LDLT recipients. Here, we present the baseline data from the study.

Methods: H2307, an ongoing 24-month (mo), multicentre (38 active sites across 14 countries), controlled study aims to randomise adult LDLT recipients in a 1:1 ratio to receive EVR (C0 3–8 ng/mL) + rTAC (C0 3–5 ng/mL) or TAC-C (6–10 ng/mL) after entering a 30-day run-in period with TAC (5–15 ng/mL) +/− mycophenolate mofetil +/− anti-IL2 induction and steroids (Figure). Randomisation is stratified based on hepatocellular carcinoma (HCC) at time of LT. HIV-negative status and known HCV and HBV status are the key inclusion criteria; exclusion criteria are HCV-negative subjects receiving transplant from HCV positive donor, MELD score >35 within 1 mo prior to transplantation, and HCC patients with extra-hepatic spread or macrovascular invasion. Study objectives are composite efficacy failure (treated biopsy proven acute rejection, graft loss or death), evolution of renal function from randomisation to month 12 and month 24, and the incidence of adverse events. Specific objectives include incidence and time to HCC recurrence, evolution of HCV viral load and allograft regeneration.

Results: Around 43 LDLT recipients (male: 67.4%, Asian: 76.7%, age <60 years: 69.8%) are enrolled to the H2307 study as of 10 December 2014. Major end stage diseases leading to transplantation are HCC (34.9%) and HBV (27.9%). At baseline, mean age was 53.1 years, eGFR (mean±SD) was 112.2±36.6 mL/min/1.73 m^2, and model end-stage liver disease score (mean±SD) was 13.3±5.0.

Conclusions: H2307 is the first randomised, multicentre study in LDLT recipients, evaluating the efficacy and safety of EVR+rTAC vs TAC-C.

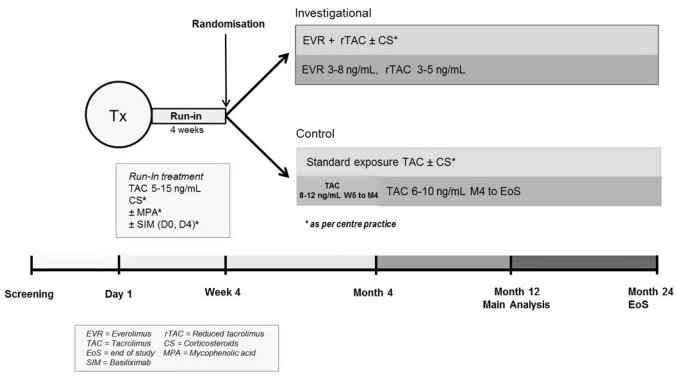

Figure: H2307 Study design.

P1340

ASSESSING NUTRITION THROUGH OBSERVATION IN EARLY CIRRHOSIS OF THE LIVER: A NURSE LED PILOT STUDY

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Background and Aims: Prevalence of malnutrition in patients with cirrhosis has been reported to be high and it has major implications on morbidity and mortality in these group of patients. Malnutrition in cirrhosis still remains underdiagnosed and undertreated in routine clinical practice.

Aims:

1. To perform nutritional assessment of patients with compensated cirrhosis using validated tools.
2. To evaluate the effect of a personalised dietary intervention on the nutritional status of patients.

Methods: The Research Nurse will identify malnourished patients from a cohort of patients with Child–Pugh A cirrhosis using a combination of handgrip strength <30 kg/F + Mid-arm muscle circumference (MAMC) <23 cm and subjective global assessment tool. All patients will undergo anthropometric measurements, blood/urine biochemistry, chronic liver disease questionnaire (CLDQ) to evaluate quality of life and Dual Energy X-ray absorptiometry (DEXA) at baseline and 6 months following the intervention.

Participants: 21 patients with Child–Pugh A cirrhosis (based on histology or combination of clinical + non invasive markers) meeting the criteria of malnutrition.

Exclusions: Liver cancer, portal/splenic vein thrombosis, on-going alcohol abuse and uncontrolled diabetes.

Intervention: Dieticians will evaluate individual's nutritional requirements which will be used to develop a personalised dietary intervention. The effect of the intervention will be evaluated at 6 months follow-up.

Primary Outcome: Improvement from baseline in the protein compartment and fat free mass on DEXA scan.

Secondary Outcomes: Improvements in CLDQ, grip strength and MAMC post intervention; compliance with dietary intervention.

Results: We will describe the prevalence of malnutrition based on the parameters that are evaluated in the study cohort. We will analyse the primary and secondary outcomes as the changes in the nutritional parameters assessed prior to and after 6 months of dietary intervention.

We will present our results at the International Liver Congress 2015.

Conclusions: This nurse-led project will highlight the importance of a multi-disciplinary approach to liver care and the relevance of nutritional assessment and dietary interventions in this specific group of patients.

P1341

PERFORM: AN IMAGING STUDY OF ARTERIAL BLOOD FLOW TO LIVER METASTASES BEFORE AND AFTER SELECTIVE INTERNAL RADIOTHERAPY

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Background and Aims: Colorectal cancer (CRC) is the third most common malignancy worldwide. The leading cause of death from this disease is progression of liver metastases. Selective internal radiotherapy (SIRT) involves the injection of yttrium-90 resin microspheres directly into hepatic arterial vessels to deliver radiotherapy to primary and secondary liver malignancies. Our research programme is focussed on drugs which can alter arterial blood flow to tumours. Since deposition of microspheres depends on flow characteristics, we decided to evaluate perfusion Computed Tomography (pCT) before and after SIRT. The aim of PERFORM is to show that imaging evaluation of arterial blood flow is an essential component of patient assessment prior to liver-directed arterial therapy.

Methods: In 58 patients due to receive systemic chemotherapy for liver metastases, and in patients due to receive the same chemotherapy plus SIRT, pCT liver scans are performed at 4 timepoints: pre-chemotherapy, after one cycle of chemotherapy (2 days before SIRT), 12 days post-SIRT and 40 days post-SIRT. A metastasis measuring 1–4 cm is selected on a pre-contrast CT study for dynamic imaging after pump-injection of iodinated contrast.

Data are analysed on GE Advantage Workstation (GE CT Perfusion 4D). Analysis involves motion correction and image co-registration
Results: At 3 participating sites, 29 participants have been recruited. Feasibility analysis of 25 pCT studies acquired from the first 7 patients treated with SIRT has shown a metastasis suitable for analysis on all studies. In 22/25 studies, dual-input pharmacokinetic modelling of tumour Blood Flow, Blood Volume, Mean Transit Time and Hepatic Arterial Fraction has been successfully performed.
Conclusions: We have demonstrated the feasibility of performing pCT scans before and after SIRT at 4 timepoints and the ability to derive reproducible data from those scans. These data will be used to measure changes in perfusion parameters after chemotherapy to optimise the delivery of SIRT. Regarding the assessment of baseline arterial flow characteristics for patient selection for SIRT, the feasibility of performing both pCT and dynamic contrast enhanced MRI at the same timepoint for exchange of information between imaging modalities will be explored.

P1342
PROSPECTIVE REAL WORLD OUTCOMES STUDY OF HE PATIENTS' EXPERIENCE ON RIFAXIMIN-α (PROSPER): AN OBSERVATIONAL STUDY AMONG 550 PATIENTS

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Background and Aims: Rifaximin-α is indicated in Europe for prevention of recurrent episodes of overt hepatic encephalopathy (HE) in patients ≥18 years old. Its efficacy was confirmed in a randomized controlled trial (Bass et al. NEJM 2010); rifaximin 550 mg b.i.d. reduced the relative risk of recurrence of HE and hospitalisation by 58% and 50%, respectively (absolute risk reduction 24% and 9%, respectively). To translate this 'efficacy' into 'effectiveness', real world data are needed. The aim of this study is to monitor the clinical effectiveness of rifaximin-α and its impact on health care resources in Europe and Australia.
Methods: This is an observational, multicentre study with 550 patients. Inclusion criteria: adults with a diagnosis of liver cirrhosis and one confirmed HE event within the past 12 months, which can include the index event. Exclusion criteria: prior treatment with rifaximin-α, contraindications to the use of rifaximin-α, hepatocellular carcinoma, inability to give informed consent or West Haven score of ≥2 at the point of study entry. Timing of recruitment: patients can enter into this study from the point of hospital discharge up to 8 weeks after hospital discharge. Study Design: patients will be followed up prospectively for 24-months. Patients will either be managed with rifaximin-α (treatment group) or not managed with rifaximin-α (control group) in the prospective phase. The treating physician will decide the allocation of treatment according to local practice. The study will also have a retrospective part, in which clinical information from medical records for the prior 12 months will be obtained. Primary endpoint: the number and duration of hospitalisation. Secondary endpoints will include mortality rate, incidence of other cirrhosis complications, 30-day readmission rate, accident and emergency visits, primary care contacts and quality of life.
Results: Initial analyses will be performed after 12 months of data collection. Full dataset should be available for analysis by July 2018.
Conclusions: Currently, there are little data on the 'effectiveness' of secondary prophylaxis and the impact on health care resource used in Europe and Australia with rifaximin-α. Therefore, the findings of this study may provide important real world evidence. Furthermore, the data could potentially provide a better understanding of the burden and natural history of HE, and variability in disease management in individual units.
PROSPER is sponsored by Norgine.

P1343
PHASE I DOSE-ESCALATION STUDY EVALUATING THE SAFETY AND PHARMACOKINETICS OF ORAL ARTESUNATE IN PATIENTS WITH ADVANCED HEPATOCELLULAR CARCINOMA (DESPARTH TRIAL)

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Background and Aims: Artemisinins are safe and effective FDA-approved antimalarial drugs. Recent studies have suggested that artemisinins also exert anti-angiogenic and cytotoxic effects on human cancer cells. Dihydro-artemisinin, the main active metabolite of artemisinins, has been demonstrated to exhibit antitumour effects toward various types of human cancers, including liver, lung, breast (clinical trial running) and pancreatic cancer. Artesunate is a water-soluble semisynthetic artemisinin with improved pharmacokinetic properties. We previously reported the antitumour effect of Artesunate in the diethylnitrosamine-induced mouse model for HCC, together with its effect on angiogenesis and multidrug resistance.
Methods: An investigator-initiated single-center phase I dose-escalation study evaluating the safety and pharmacokinetics of oral Artesunate in patients with advanced HCC was designed and is now recruiting. Advanced HCC patients with Child–Pugh class not higher than B7 are included when sorafenib is stopped due to intolerance or therapeutic failure or when sorafenib is contraindicated or refused. Approximately 16 patients will be enrolled in this trial.
The first patient will receive 200 mg Artesunate once-daily for 14 days. If no dose-limiting toxicity (DLT) is observed after 14 days, the next patient will start at a daily dose of 300 mg Artesunate. If no DLT is observed after 14 days, a cohort of 3 patients will receive 400 mg once-daily for 14 days. For each subsequent cohort of 3 patients 200 mg will be added to the dose, until maximum tolerated dose (MTD) is determined. Treatment will continue until both radiologic and symptomatic progression are reached, adverse reactions requiring discontinuation of study treatment occur, or mortality.
Plasma concentrations of Artesunate will be determined by ultra-performance liquid chromatography tandem mass-spectrometry. Pharmacokinetic parameters that will be measured are C_{max}, C_{min}, T_{max}, and AUC.
Results: The primary objectives are to assess the safety, estimate the MTD, and characterize the pharmacokinetics of Artesunate in HCC patients with mild impaired liver function.
The secondary objectives are to evaluate the time to tumor progression, overall survival and quality of life based on the Functional Assessment of Cancer Therapy Hep-30 scale.
Conclusions: Since Artesunate could represent a novel drug in the treatment of advanced HCC, determination of the MTD in this patient population is primordial.

P1344

DETERMINING THE HEPATIC CYST PENETRATION OF CEFAZOLIN AND FACTORS AFFECTING PENETRATION, AN EXPLORATIVE STUDY

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Background and Aims: Hepatic cysts are fluid-filled cavities located in the liver parenchyma. With aspiration sclerotherapy (AS) hepatic cysts are drained and flushed with a sclerosing agent. AS results in cyst volume reduction and is indicated in patients with a symptomatic dominant hepatic cyst. Cyst infection, either spontaneous or following AS, is a severe complication as it frequently requires hospitalization, long-term antibiotics or invasive treatment. Cefazolin prophylaxis is given to prevent cyst infection following AS. Evidence that antibiotics reach adequate intracystic concentrations is lacking. Our primary objective is to determine cefazolin penetration into hepatic cysts. Secondary objective is to identify factors affecting antibiotic penetration and to evaluate the association between antibiotic penetration and development of AS-induced cyst infection.
Methods: We aim to include nine subjects (aged ≥18 years) who will be treated by AS. Prior to AS, patients receive a single dose of cefazolin (intravenous infusion 1000 mg in 1 hour). We will collect blood following cefazolin infusion to determine the peak cefazolin blood concentration (μg/ml). The cyst aspirate concentration of cefazolin (μg/ml) will be measured in cyst fluid obtained during AS. Simultaneously, we will perform a second blood withdrawal during AS to determine the blood concentration of cefazolin (μg/ml). Cyst aspirate and blood concentrations of cefazolin will be measured using high-performance liquid chromatography (HPLC). Primary endpoint is hepatic cyst penetration of cefazolin defined as the ratio (%) of cyst aspirate concentration (μg/ml) to blood concentration (μg/ml) of cefazolin. Secondary study parameters are location and volume of the aspirated hepatic cyst, cyst fluid and blood parameters, and development of clinical signs indicating AS-induced cyst infection.
Conclusions: This study investigates the hepatic cyst penetration of cefazolin and factors that affect cyst penetration.

P1345

A RANDOMIZED, OPEN-LABEL STUDY TO EVALUATE EFFICACY AND SAFETY OF OMBITASVIR/PARITAPREVIR/RITONAVIR CO-ADMINISTERED WITH RIBAVIRIN IN ADULTS WITH GENOTYPE 4 CHRONIC HEPATITIS C INFECTION AND CIRRHOSIS

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Background and Aims: Hepatitis C virus (HCV) infection is a global health problem, with over 170 million individuals chronically infected worldwide. HCV genotype 4 (GT4) represents approximately 20% of global HCV infection. Although GT4 infection is more common in the Middle East and sub-Saharan Africa, with globalization, GT4 is now seen in many other countries. Combinations of direct-acting antiviral agents (DAAs) targeting different steps of viral replication have the potential to significantly improve HCV treatment. In a Phase 2b study (PEARL-I), a sustained virologic response rate 12 weeks post-treatment (SVR12) of 100% was achieved among both treatment-naïve and prior peginterferon (pegIFN) and ribavirin (RBV) experienced HCV GT4-infected patients without cirrhosis. These patients were treated for 12 weeks with the 2DAA IFN-free regimen of ombitasvir (OBV), a NS5A inhibitor, plus paritaprevir (formerly ABT-450, a NS3/NS4A protease inhibitor, identified by AbbVie and Enanta co-dosed with ritonavir [PTV/r]) and RBV. Furthermore, a Phase 3 study (TURQUOISE-II) in HCV GT1-infected patients with cirrhosis demonstrated high efficacy and good tolerability of OBV/PTV/r with dasabuvir and RBV. The current study will evaluate the efficacy and safety of co-formulated OBV/PTV/r with RBV in HCV GT4-infected patients with compensated cirrhosis.
Methods: This Phase 3, randomized, open-label, multinational study (NCT 02265237) will enroll 160 HCV GT4-infected treatment-naïve or IFN/RBV or pegIFN/RBV (pegylated-interferon alfa-2a or alfa-2b) treatment-experienced patients with compensated cirrhosis. Patients are randomized 1:1 to receive co-formulated OBV/PTV/r co-administered with weight based RBV for 12 or 16 weeks. The primary objectives of the study are to assess the safety of the 2DAA regimen and to compare the proportion of patients achieving SVR12 (HCV RNA <lower limit of quantification) with the 2DAA regimen to the historical SVR12 rate for HCV GT4-infected patients treated with pegIFN/RBV. The secondary objectives are to compare SVR12 rates between the 12 and 16 week treatment arms, and to assess in each treatment arm the percentage of patients with on-treatment virologic failure and the proportion of patients with post-treatment relapse within 12 weeks following the end of treatment. The study will also include exploratory pharmacokinetic, resistance and pharmacogenomics analyses. The study opened in October 2014 and is currently enrolling patients in North America and Europe.

P1346

STUDY PROTOCOL FOR A PARTLY RANDOMISED, OPEN-LABEL PHASE IIA TRIAL OF ONCE-DAILY SIMEPREVIR COMBINED WITH SOFOSBUVIR FOR THE TREATMENT OF HCV GENOTYPE 4-INFECTED PATIENTS WITH OR WITHOUT CIRRHOSIS (OSIRIS)

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Background and Aims: HCV genotype 4 is mainly found in the Middle East and Africa, but it is beginning to spread to Europe. Although direct-acting antiviral agents are revolutionising treatment of hepatitis C, HCV genotype 4-infected patients have been under-represented in large multicentre clinical studies. Simeprevir is an inhibitor of HCV NS3/4A protease effective against HCV genotype 4. Sofosbuvir is a nucleotide NS5B polymerase inhibitor also effective against HCV genotype 4. This trial will evaluate efficacy and safety of 8 or 12 weeks' treatment with once-daily simeprevir and sofosbuvir for HCV genotype 4-infected patients in Egypt.
Methods: OSIRIS (Optimize Simeprevir In a Regimen Including Sofosbuvir), is a Phase IIa, partly randomised, multicentre open-label trial (NCT02278419). Sixty HCV genotype 4-infected, treatment-naïve or experienced adult patients with baseline HCV RNA >10,000 IU/mL and compensated liver disease will be enrolled.

Forty patients without cirrhosis will be randomised (1:1) to receive oral simeprevir (TMC435) 150 mg qd combined with sofosbuvir 400 mg qd for either 8 (Group A1) or 12 weeks (Group A2) (Figure). Randomisation will be stratified by prior treatment experience and METAVIR score (Stratum 1: treatment naïve and F0–F2; Stratum 2: treatment-experienced and/or F3). A further 20 patients with cirrhosis will receive the regimen for 12 weeks (Group B).

The primary objective is to determine the efficacy of this regimen, assessed by the percentage of patients with HCV RNA IL28B genotype, and demographic/baseline characteristics. The lower limit of the 95% confidence interval (CI) of the overall SVR12 rate will be compared against composite historical control rates; superiority will be concluded if the lower limit of the 95% CI is greater than the threshold. Secondary objectives include SVR at 4 and 24 weeks after end of treatment; relative efficacy of 8 versus 12 weeks' treatment; on-treatment virologic responses; frequency of treatment failure and relapse; and safety and tolerability of the regimen.

Conclusions: This study will evaluate the efficacy and safety of a once-daily regimen comprising simeprevir 150 mg qd plus sofosbuvir 400 mg qd for treatment of patients with HCV genotype 4 infection.

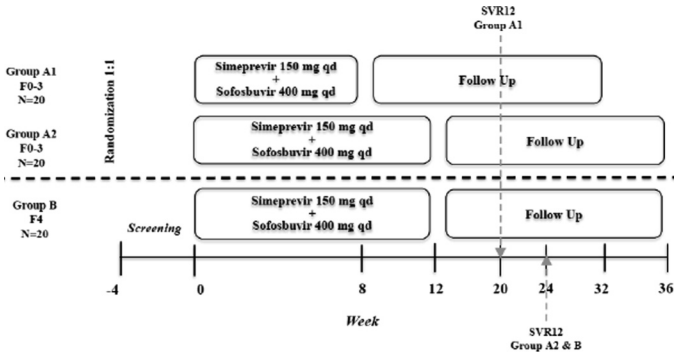

Figure: Randomisation. F0–3 and F4 refer to METAVIR fibrosis score. qd, once daily; SVR12, sustained virologic response at 12 weeks after end of treatment.

P1347

A PROSPECTIVE, MULTI-CENTRIC STUDY ABOUT DISEASE DEVELOPMENT FOR LIVER CIRRHOSIS PATIENTS WITH ACUTE DECOMPENSATION OR NON-CIRRHOTIC PATIENTS WITH ACUTE HEPATIC INJURY

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Background and Aims: Acute-on chronic liver failure (ACLF) is a distinct entity encompassing the acute deterioration of liver function culminating in multiple organ failure and high short-time mortality. Our Chinese single center 890 HBV cirrhotic patients with acute decompensation (AD) cohort data which be collaborated with and analyzed by CANONIC study group has been shown both HBV and alcoholic related ACLF belong to a homogenous disease entity. However, that conclusion still need to be confirmed by a prospective multi-centric study in HBV prevailing region.

Methods: 13 Chinese national wide liver centers which total beds are around 500 have been included. Continuous hospitalized patients with any etiology induced chronic liver disease (including both cirrhotic and non-cirrhotic) with AD or acute hepatic injury (aminotransferase >3NL) will be recruited from January to December 2015. Biochemical parameters, organ failure, mortality and liver transplantation rate will be collected at days 1, 4, 7, 14, 21 and 28 after enrollment. The patients will be continuously followed up once a month until the 24th month after hospital discharge. Because expecting for lacking of liver biopsy in most of the patients, both CT and FibroScan will be used for differentiating non-cirrhotic patients, especially for hepatitis with mild fibrosis patients from cirrhotic patients.

Results: 2500–3000 patients are planed to be recruited and followed-up in this study. Two different cohorts for ACLF patients with cirrhosis and acute deterioration patients with mild liver fibrosis (FibroScan value <8.5) will be identified. Disease severity, organ failure score and mortality will be compared between ACLF and non-ACLF cirrhotic patients (according to CLIF-C-OF score), or between ACLF cirrhotic and mild fibrosis with acute deterioration patients. The homogeneity for different etiological ACLF cirrhotic patients will be statistically calculated. Whether mild fibrosis with acute deterioration patients belong to the same disease entity with ACLF cirrhotic patients or to be a new disease entity will be identified in the study.

Conclusions: Different etiology related ACLF belong to a same disease entity and could share an universal diagnostic criteria, severity classification and prognostic models will be confirmed by this prospective multi-centric study. Whether non-cirrhotic patients with acute deterioration is a separated disease entity or a homogeneous entity with ACLF cirrhotic patients will be identified in this study.

P1348

RCT FOR ANTI-HEPATOFIBROTIC EFFECT OF A HERBAL DRUG (CGX)

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Background and Aims: The progress of hepatic fibrosis determines the clinical outcome of patients, but no therapeutics for this disease exists yet. CGX is a modification of a traditional Korean herbal medicine, which has a hsitory of the long clinial use for patients with various chronic liver inuries and multiple pharmacological results on anti-hepatofibrosis in animal models. CGX is now under clinical trial phase III for anti-hepatofibrosis therapeutic effect in South Korea.

Methods: The clinical study is a phase III trial of stratified randomized (intake antiviral drugs vs. no use), placebo controlled and double blind. 174 patients with chronic HBV, HBV or alcoholic injury are enrolled in 2 hospitals. All patients should have a moderate fibrosis score by 7< and 17 kPa of LSM using Fibroscan. They should not be in advanced fibrosis or cirrosis (ascites, esophageal varix, TB >3 mg/dl, AST, ALT > ULN > 5 folds, INR >2.0 or platelets <80,000/mm^3, BMI >30). Patients take a placebo or CGX (1 or 2 gram daily) for six months. The primary outcome is the changed value of LSM during 6 months. The secondary measurement includes the changed values of hyaluronic acid (HA), serum TGF-β1 and PDGF, AST to platelet ratio index (APRI), and QOL (SF-36) respectively.

Results: This RCT will be continue until June 2015 (From June 2013; RCT registration number is KCT0000773).

Conclusions: The objective of the present study is to present the overall status of a clinical evaluation for CGX, an herbal drug-derived anti-hepatofibrotic.

P1349

RANDOMISED PLACEBO-CONTROLLED TRIAL ASSESSING WHETHER PROBIOTIC SUPPLEMENTS AMELIORATE DEPRESSIVE SYMPTOMS IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION

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Background and Aims: Patients with chronic hepatitis C virus (HCV) infection frequently present with co-morbid depression. The pathophysiology of depression is multifactorial, and intestinal microbiota have been reported to influence depressive symptoms. Studies in mice report specific strains of gut bacteria to positively influence brain neurotransmitters implicated in depression, such as GABA. A randomised placebo-controlled trial reported a probiotic supplement to alleviate psychological distress and depressive symptoms in otherwise healthy individuals (Messaoudi et al. Br J Nutr 2011).
Study aim: Given the high prevalence of depressive symptoms in patients with chronic HCV infection, this study assesses whether oral administration of select commensal bacteria modulates depressive symptoms and alters intestinal flora architecture in favour of increased lactobacillus and bifidobacterium in these patients. We hypothesised that probiotic supplementation reduces the severity of depressive symptoms.
Methods: A randomised, double-blind placebo-controlled study is being carried out in adults with chronic HCV infection and depression, that have been awaiting new DAA-based antiviral therapies. The intervention product contains two strains of lactic acid bacteria *Lactobacillus helveticus* and *Bifidobacterium longum*. Patients are randomly given the probiotic or identical placebo orally for 60 days. Patients are assessed using a validated self-report questionnaire (Beck Depression Inventory-II) for depressive symptoms at baseline, and after 30 and 60 days of treatment. Fecal samples collected during these time points enable us to characterise the microbial community using 16S rRNA pyrosequencing and to assess for changes in the gut microbiota in parallel to changes in depressive symptoms. Body composition using bioelectrical impedance analysis and other potential confounders such as dietary intake and physical activity are captured during all study time points, using food diaries and actigraphs.

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LIVER AND SPLEEN STIFFNESS MEASUREMENT FOR PREDICTING VARICES BLEEDING AND PROGNOSIS IN RUSSIAN PATIENTS WITH HCV AND ALCOHOL RELATED CIRRHOSIS

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Background and Aims: Hepatitis C viral infection and alcohol are the main ethiological factors of liver cirrhosis in Russia. The direct measurement of hepatic venous pressure gradient is the reference standard for detection of level of portal hypertension but it is expensive and invasive. Colecchia et al. in 2012 published a predictive model for HVPG values that included spleen (SS) and liver stiffness (LS) measured by transient elastography. In our study we tried to modify and study diagnostic test accuracy of this model in a Russian cohort of patients.
The aim of the study is to establish the diagnostic test accuracy of non-invasive liver and spleen stiffness measurement model for prognosis of bleeding from esophageal varices in HCV and alcohol-related cirrhosis.
Methods: A cohort of 100 consecutive patient with HCV and alcohol-related cirrhosis will be enrolled in the study and divided into two groups based on ethiology. After the study design: baseline blood test sampling, upper endoscopy for detecting esophageal varices, ultrasound, liver stiffness measurement with Fibroscan (LS), spleen stiffness measurement with Fibroscan (SS) will be performed at the same day. Abstinence will be recommended to all patients with alcoholic cirrhosis. HCV patients who meet inclusion criteria will be treated in clinical trials with different interferon-free AVT regimes. All baseline procedures will be repeated in 6–12 month after follow up. We register all clinical outcomes during follow-up period.
In this paper we report the data from 22 enrolled patients (15 patients with HCV-related cirrhosis, 7 patients with alcoholic cirrhosis).
Results: LS in the group with varices is significantly higher than in the group without ($p = 0.005$). Median of LS in varices group is 29.5 kPa (IQR 25–48) vs 18.5 kPa (IQR 16.9–21.8). No statistically significant differences between two groups when competed SS were found (small number of enrolled patients). Nevertheless the level of portal vein pressure calculated using the predictive model is significantly higher in varices group – 16.8 (IQR 11–23) vs 9.4 (IQR 7–13) respectively ($p = 0.009$). In addition a statistically significant difference observed when comparing LS in patients with alcohol-related cirrhosis [median LS 48 kPa (IQR 25–75)] with HCV-related group [median LS 21 kPa (IQR 16–27); $p = 0.004$].

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AN OPEN-LABEL STUDY TO EVALUATE THE EFFICACY AND SAFETY OF CO-FORMULATED OMBITASVIR/PARITAPREVIR/RITONAVIR WITH RIBAVIRIN IN ADULTS WITH CHRONIC HCV GENOTYPE 4 INFECTION IN EGYPT

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Background and Aims: Hepatitis C virus (HCV) genotype (GT) 4 is responsible for almost 90% of HCV infections in Egypt. Although HCV GT4 infection is more common in the Middle East and sub-Saharan Africa, the prevalence of GT4 is increasing in Europe. The efficacy and safety of the direct acting antiviral agents (DAA) ombitasvir (OBV), a NS5A inhibitor and paritaprevir (formerly ABT-450, a NS3/4A protease inhibitor, identified by AbbVie and Enanta, co-dosed with ritonavir, PTV/r) with or without ribavirin (RBV) were studied in 135 patients with HCV GT4 infection without cirrhosis in the multicenter Phase 2b study, PEARL-I. SVR12 was 100% in both treatment naïve and prior interferon (IFN) and RBV experienced patients receiving OBV plus PTV/r with RBV for 12 weeks. The current trial will be the first to evaluate OBV/PTV/r with RBV in an exclusively Egyptian population of patients with and without cirrhosis.
Methods: This is a phase 3, open-label, multicenter study (NCT02247401). Approximately 160 treatment-naïve and prior pegIFN/RBV experienced patients with HCV GT4 infection will be enrolled at approximately 5 sites in Egypt. The enrolled population will consist of 100 patients without cirrhosis (50 treatment-naïve and 50 treatment-experienced) and 60 patients with compensated cirrhosis (at least 20 treatment-experienced patients). All non-cirrhotic patients will receive co-formulated OBV/PTV/r once-daily (25 mg/150 mg/100 mg) with weight based RBV for 12 weeks. Cirrhotic patients will be randomized 1:1 to OBV/PTV/r with weight based RBV for 12 or 24 weeks. All patients will be followed for 48 weeks after end of treatment. The primary objective of this

study is to assess the efficacy (proportion of patients with HCV RNA <lower limit of quantification 12 weeks after last dose of study drug) and safety of OBV/PTV/r with RBV among treatment-naïve and prior pegIFN/RBV experienced HCV GT4-infected patients without cirrhosis or with compensated cirrhosis. Secondary objectives of this study are to assess the proportions of patients in each treatment arm with on-treatment virologic failure and with post-treatment relapse within 12 weeks following the end of treatment. The study opened in October 2014.

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EVENTS 2015

LIVER DISEASE IN RESOURCE LIMITED SETTINGS

Monothematic Conference
Bucharest, Romania
May 29-30

The EASL Monothematic Conference on Liver diseases in resource-limited settings is a very timely conference due to the greater than ever strain on healthcare budgets, the high incidence of liver diseases in resource-limited regions, and the fantastic new drug treatments that come at an incredible cost.

It is intended for those interested in bringing better care for liver patients to areas in need, and will be a time to discuss problems and find solutions on this topic.

The faculty will be a mix of high level liver experts as well as representatives from governmental and non-governmental organisations.

LIVER CIRRHOSIS AND COMPLICATIONS

Clinical School of Hepatology Course 24
Moscow, Russia
June 5-6

AUTOIMMUNE HEPATITIS

Monothematic Conference
London, United Kingdom
September 3-5

ADDRESSING END-STAGE LIVER DISEASE - A MULTIFACETED CHALLENGE

Special Conference
Glasgow, United Kingdom
September 25-27

MANAGEMENT OF CRITICALLY ILL CIRRHOTIC PATIENT

Clinical School of Hepatology Course 25
Barcelona, Spain
October 22-23

EASL MASTERCLASS

Milan, Italy
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EVENTS 2016

T-CELL RESPONSES IN VIRAL HEPATITIS AND HEPATOCELLULAR CARCINOMA: FROM IMMUNOBIOLOGY TO NOVEL THERAPEUTIC APPROACHES
Basic School of Hepatology Course 11
Freiburg, Germany
February 5-6

EASL HCC SUMMIT
Geneva, Switzerland
February 11-14

THE INTERNATIONAL LIVER CONGRESS™ 2016
Barcelona, Spain
April 13-17

MANAGEMENT OF METABOLIC DISORDERS ON LIVER DISEASE
Monothematic Conference
Riga, Latvia
May 13-14

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VIRAL HEPATITIS
Clinical School of Hepatology Course 26
Ankara, Turkey
June 3-4

LIVER FIBROSIS: THE NEXT GOAL OF TARGETED THERAPY? OR FROM BASIS TO BEDSIDE, WHERE DO WE STAND?
Monothematic Conference
Porto, Portugal
June 17-18

NEW PERSPECTIVES IN HEPATITIS C VIRUS INFECTION - THE ROADMAP FOR CURE
Special Conference
Oslo, Norway
September 15-17

UNMET NEEDS IN HEPATOLOGY
Clinical School of Hepatology Course 27
Paris, France
October 27-28

NUTRITION IN LIVER DISEASE
Monothematic Conference
Ljubljana, Slovenia
November 24-26

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